ANNUAL RESEARCH PROGRAM REPORT

(FY 2006)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

NUTRITIONAL DETERMINANTS OF HEALTH

MANAGEMENT UNIT

5450-10-00

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: DAVID M KLURFELD **Principal Investigator:** JACK T SAARI

Start Date: 07/21/2004 **Term Date:** 04/30/2009

National Programs: 107 N Human Nutrition

Title: DIETARY COPPER REQUIREMENTS FOR OPTIMAL CARDIOVASCULAR FUNCTION AND HEALTH

Period Covered From: 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter?**

Cardiovascular disease is the leading cause of death in this country, with the direct annual cost projected to approach $250 billion in 2005. Basic research and epidemiological studies have indicated that inadequate dietary intakes of mineral elements such as calcium, copper, magnesium, zinc, as well as overload of iron are associated with altered functions of the heart and circulation. Furthermore, dietary surveys indicate that appreciable numbers of people have sub-optimal intakes of at least some of these minerals. However, we presently have little definitive proof that changing dietary practices with regard to these minerals will benefit cardiovascular health. A clear understanding of how these minerals, particularly at marginal intakes, contribute to cardiovascular function will provide the basis for dietary recommendations that improve the health of the general public. The current project will focus on the contribution of dietary copper to cardiovascular health.

The overall objective is to determine, using animal models, whether copper (Cu) intakes consistent with those observed in humans can adequately support cardiovascular functions. This objective will be pursued through the mechanistic tether of oxidative stress/altered nitric oxide metabolism by which Cu functions, and includes the following specific objectives: 1) to develop a strategy for assessment of marginal copper deficiency in animals; to use this strategy to determine biomarkers of copper status that are suitable for assessment of marginal status in humans, 2) to determine the contribution of oxygen- and nitrogen-derived reactive species to the cardiomyopathy (metabolic, contractile) induced by Cu deficiency, and the dietary intakes at which this pathology occurs, 3) to determine whether low Cu intakes consistent with those observed in humans can impair nitric oxide-dependent control of blood vessels and blood pressure regulation, 4) to determine whether the oxidative stress induced by Cu deficiency affects homocysteine metabolism and, thereby, cardiovascular function, and whether such effects influence nitric oxide-dependent signal transduction and/or other mechanisms that affect atherosclerosis, 5) to determine whether marginal Zn deficiency can exacerbate or unmask cardiovascular effects of sub-optimal Cu status by virtue of its role in oxidative/nitrosative metabolism.

Research will address components of National Program 107, Human Nutrition (100%). From component 1, Nutrition Requirements, objectives A (Biomarkers), B (Mechanism of Action), C (Nutrient Interactions), E (Genetic Variability) and G (Function and Performance) will be addressed. From component 2, Diet, Genetics, Lifestyle, and the Prevention of Obesity and Disease, objective A (Identify nutritional, environmental and genetic factors that modify the effects of nutrient intake and metabolism on health outcomes) will be addressed.

Project Number: 5450-51000-038-00D Accession: 0408766 FY: 2006

2. List by year the currently approved milestones (indicators of research progress)

Year 1 (FY 2005)

Assess relationships between organ copper and marginal intakes of dietary copper; select organ with best discrimination of copper intake.

Determine if copper deficiency causes reduced mitochondrial respiratory complex activity.

Identify mitochondrial respiratory complexes causing increased hydrogen peroxide production during copper deficiency.

Determine nitric oxide effect on mitochondrial respiration in copper deficiency.

Determine effect of copper deficiency on homocysteine metabolism.

Year 2 (FY 2006)

Determine signaling pathway for induction of inducible nitric oxide synthase during copper deficiency.

Determine nature of effect of altered nitric oxide on blood pressure during copper deficiency.

Determine effect of copper deficiency on bilirubin and biliverdin reductase.

Determine if low zinc acts to exaggerate cardiovascular effects of marginal copper.

Year 3 (FY 2007)

Correlate organ copper content with semi-direct indicators of copper status; select best potential single biomarker(s).

Identify respiratory complexes affected by developmental copper deficiency.

Determine extent of oxidative modification of mitochondrial DNA by copper deficiency.

Determine role of nitric oxide in impaired contractile function of copper deficiency.

Determine relationship between nitric oxide, oxidative stress, homocysteine in atherosclerotic symptoms of copper deficiency.

Clarify role of oxidative/nitrosative stress in zinc/copper interaction.

Year 4 (FY 2008)

Determine nature of effect of altered nitric oxide on coronary vessels during copper deficiency.

Determine role of heme oxygenase in atherosclerotic effects of copper deficiency.

Determine the ability of a diet high in antioxidants to ameliorate oxidant stress

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2006

induced by low copper/low zinc. [This is a new milestone (positive contingency) that anticipates the finding of a role for oxidative/nitrosative stress in the detrimental interaction between zinc and copper.]

Year 5 (FY 2009)

Correlate organ copper content with combinations of indicators of copper status; select best combination of indicators as biomarker.

Identify oxidized, nitrated mitochondrial proteins in copper deficiency.

Identify mitochondrial DNA mutations of copper deficiency.

Relate mitochondrial DNA mutations to expression of respiratory complexes caused by copper deficiency.

Determine whether elevation of inducible nitric oxide synthase preconditions copper-deficient hearts [contingency].

3a. List the milestones (from the list in Question 2) that were scheduled to be addressed in FY 2006.

1. Determine signaling pathway for induction of inducible nitric oxide synthase during copper deficiency.

   Milestone Substantially Met

2. Determine nature of effect of altered nitric oxide on blood pressure during copper deficiency.

   Milestone Not Met

   Reason not met: Other

3. Determine effect of copper deficiency on bilirubin and biliverdin reductase.

   Milestone Not Met

   Reason not met: Critical SY Vacancy

4. Determine if low zinc acts to exaggerate cardiovascular effects of marginal copper.

   Milestone Substantially Met

5. Milestone from 2005 met in 2006: Determine effect of copper deficiency on homocysteine metabolism.

   Milestone Substantially Met

3b. List the milestones (from the list in Question 2) that you expect to address over the next 3 years (FY2007, 2008 & 2009). What do you expect to accomplish, year by year, over the next three years under each milestone?

Year 1 (FY 2007)

Correlate organ copper content with semi-direct indicators of copper status; select best potential single biomarker(s).
Due to a critical SY vacancy, this milestone will not be completed.

Identify respiratory complexes affected by developmental copper deficiency.
Respiratory complex activities and protein subunits of complex I and complex IV will be assayed in the hearts of neonates from marginally Cu-deficient dams at various

ages up to one year. IMPACT: Parallel reductions in complex activities and protein subunit content in cardiac mitochondria indicate that marginal Cu intakes during pregnancy leads to prolonged impairment of mitochondria function in the first generation and increases risk for developing age-related cardiac disease.

Determine extent of oxidative modification of mitochondrial DNA by copper deficiency.

The content of 8-hydroxydeoxyguanosine will be measured in heart mitochondrial DNA from rats fed diets containing deficient, marginal, and adequate levels of copper. IMPACT: Increased 8-hydroxydeoxyguanosine indicates that mitochondrial DNA can be oxidatively modified during Cu deficiency. This can cause mutations in mitochondrial DNA that permanently impair mitochondrial function and lead to cardiac disease.

Determine role of nitric oxide in heart contractile function.

Due to a critical SY vacancy, this milestone will not be completed.

Determine relationship between nitric oxide, oxidative stress, homocysteine in atherosclerotic symptoms of copper deficiency.

Due to a critical SY vacancy, this milestone will not be completed.

Clarify role of oxidative/nitrosative stress in zinc/copper interaction.

If it is determined that a moderate zinc deficiency exacerbates signs of marginal copper deficiency, an experiment with rats will be performed to determine whether cardiovascular effects are modified by changing nitric oxide formation. IMPACT: If, in the year 2006 study, zinc proves to have an effect on copper-dependent alteration of cardiovascular function, a nitric oxide mechanism will be tested to further our knowledge of the molecular events affected by these two nutrients.

Year 2 (FY 2008)

Determine nature of effect of altered nitric oxide on coronary vessels during copper deficiency.

Due to a critical SY vacancy, this milestone will not be completed.

Determine role of heme oxygenase in atherosclerotic effects of copper deficiency.

Rats of varying copper status will be subjected to carotid artery damage and then treated with inhibitors of heme oxygenase (metalloprotoporphyrins) to determine the extent to which this enzyme plays a role in the atherosclerotic effects of dietary copper deficiency. IMPACT: This study will further delineate the mechanism of action of low dietary copper in impaired blood vessel function.

Determine the ability of a diet high in antioxidants to ameliorate oxidant stress induced by low copper/low zinc:

If it is determined that combined moderate zinc and copper deficiencies results in an elevated oxidant stress that adversely affects cardiovascular health, an experiment with rats will be performed to determine whether antioxidants (e.g., vitamin C, vitamin E) ameliorate the oxidant stress. IMPACT: The study should help define conditions under which low dietary copper may adversely affect cardiovascular health.

Year 3 (FY 2009)

Correlate organ copper content with combinations of indicators of copper status;

select best combination of indicators as biomarker.

Due to a critical SY vacancy, this milestone will not be completed.

Identify oxidized and nitrated mitochondrial proteins in copper deficiency.

Mitochondria will be isolated either from hearts of rats fed Cu-deficient and marginally Cu-deficient diets or from hearts of the offspring of dams that were marginally Cu-deficient during pregnancy and lactation. Oxidized and nitrated proteins will be identified by immunoblotting using antibodies that recognize 2,4-dinitrophenylhydrozone derivatives of protein carbonyls and nitrotyrosine. IMPACT: This study will identify mitochondrial proteins in cardiac mitochondria that are oxidized or nitrated as a result of direct dietary Cu deficiency and as a result of Cu deprivation during pre-or postnatal development. Ascertaining the identity of the modified proteins will assist in pinpointing which mitochondrial metabolic alterations contribute to heart disease caused by Cu deficiency.

Identify mitochondrial DNA mutations of copper deficiency.
Relate mitochondrial DNA mutations to expression of respiratory complexes caused by copper deficiency.

Heart mitochondrial DNA from the adult, Cu-repleted, offspring of dams that were marginally Cu-deficient during pregnancy and lactation will be examined for deletion mutations by semi-qualitative polymerase chain reaction. If such mutations occur, the extent of their effect on the expression of mitochondrial respiratory complex proteins will be determined by immunoblotting. IMPACT: An increase in the occurrence of mutations in cardiac mitochondrial DNA and resultant changes in the expression of respiratory complex proteins indicates that Cu-deficiency during pre- and postnatal development promotes the development of age-related heart disease.

[Determine whether elevation of inducible nitric oxide synthase preconditions copper-deficient hearts (contingency).]

Due to a critical SY vacancy, this milestone will not be completed.

4a. List the single most significant research accomplishment during FY 2006.

Reduced cardiac respiratory enzyme activity in the offspring of moderately Cu-deficient rats results from low dietary Cu during pregnancy, not during lactation:

Our previous studies showed that low Cu intake by rats during pregnancy and lactation reduced cytochrome c oxidase activity in cardiac mitochondria of the offspring. The reduction in enzyme activity was first observed late in the lactation phase of heart development and persisted in the offspring even after nine months of repletion with adequate dietary Cu. To determine whether this defect originated before or after birth, pups from moderately Cu-deficient dams were nursed by Cu-adequate dams and pups from Cu-adequate dams were nursed by moderately Cu-deficient dams from one day after birth. This cross-fostering did not reverse the reduction of heart cytochrome c oxidase activity in pups from Cu-deficient dams nor did it cause a defect in those from Cu-adequate dams. This indicates that the long-term loss of cytochrome c oxidase activity in the offspring of moderately Cu-deficient dams is caused by a defect in heart development before birth. IMPACT: Dietary surveys indicate that pregnant women often do not meet the current recommended daily intake for dietary Cu. Our finding suggests that moderately low Cu intake by pregnant women could cause a developmental defect in the fetal heart that may increase the risk of heart disease in their children, particularly as the children become older, because of long-term impairment of mitochondrial function. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and

new classes of nutrients in the food supply and provide that information in databases.]

4b. List other significant research accomplishment(s), if any.

Copper deficiency lowers plasma homocysteine and affects mRNA expression of several enzymes involved in homocysteine/methionine metabolism:

Homocysteine was found to be decreased in copper deficient rats. Homocysteine, a risk factor in cardiovascular disease, can be metabolized (removed) in one of two ways. It can enter the irreversible transsulfuration pathway or it can be remethylated to form methionine. Our data suggest the remethylation of homocysteine by methionine synthase is increased in copper deficient rats. Further, mRNA for methylenetetrahydrofolate reductase, an enzyme that makes one of the substrates for methionine synthase, is also increased in copper deficiency. IMPACT: These results are contrary to published results and suggest that the risk of heart disease by copper deficiency is not mediated by elevation of homocysteine. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.]

Dietary copper deficiency selectively reduces genetic expression of only one of five respiratory enzyme complexes in the heart:

Dietary copper (Cu) deficiency is known to impair mitochondrial respiratory function, which is catalyzed by five membrane-bound multiple protein complexes. However, few studies have simultaneously analyzed the effect of Cu deficiency on the subunit protein expression of all five protein complexes. Examination of expression of subunits in each of the five respiratory complexes in Cu-deficient rat hearts revealed that only in the cytochrome c oxidase complex was the subunit expression reduced. IMPACT: This study further characterizes the defect in the ability of the heart to use oxygen under Cu-deficient conditions and thus contributes to our knowledge of heart failure in dietary Cu deficiency. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.]

Dietary Cu deficiency increases nitric oxide production in rat hearts:

Nitric oxide is a signaling molecule that, depending on how it is produced, can be either harmful or beneficial to heart function. We found that two enzymes that produce nitric oxide, one that initiates a pathway that leads to cell death and the other that leads to cell protection, are both elevated in dietary copper deficiency in rats. IMPACT: These findings contribute to the view that dietary copper deficiency promotes both heart failure and actions that compensate for this failure and suggest signaling mechanisms by which this may take place. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.]

4c. List significant activities that support special target populations.

None.

Project Number: 5450-51000-038-00D Accession: 0408766 FY: 2006

4d. Progress report.

None.

5. Describe the major accomplishments to date and their predicted or actual impact.

Long-term marginal copper deficiency in adult animals causes heart and blood vessel pathology:

Dietary surveys indicate that many humans consume less than the recommended amount of copper. Although studies in animals indicate that dietary copper deficiency causes defects in cardiovascular (heart and blood vessel) structure and function, copper intake in these studies is generally too low to be relevant to human consumption. In a collaborative study with the University of Louisville, scientists at the GFHNRC found that if adult rats are fed marginally-deficient levels of copper, but for sufficiently long periods of time, they too exhibit defects in cardiovascular function and structure that are similar to those observed in severely-deficient young rats. IMPACT: These findings, reported in two papers (Li et al, 2005; Falcone et al, 2005), illustrate that low copper intakes in animals that are comparable to those observed in humans can impair heart and blood vessel function. This provides a rationale for testing for such a possibility in humans. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.]

Pinto beans provide a bio-available source of dietary copper:

Dry edible beans have been shown to be beneficial to cardiovascular health, but the active ingredient(s) for this benefit is(are) unknown. Because copper is known to be essential for cardiovascular health and beans are known to be a good source of copper, we hypothesize that copper from beans could support cardiovascular health. In order to determine this, we first tested whether the copper from dry edible beans was bio-available. We found that when copper was fed to copper-deficient rats in the form of pinto beans, it was equally as effective as inorganic copper in restoring copper status indices (e.g., organ copper, activity of copper-dependent enzymes) to those rats. IMPACT: These findings indicate that the bean's structure does not impede the delivery of its copper to the animal, which paves the way for testing of effectiveness of bean copper on cardiovascular function in both animals and humans. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients in the food supply and provide that information in databases.]

Copper deficiency causes elevation of a structural elastic protein in the heart:

We have demonstrated for the first time an 85% increase in fibulin-5 (also known as DANCE/EVEC) and a 71% decrease in cytochrome C oxidase (CCO) VIb subunit, but no change in succinate dehydrogenase complex (also known as complex II) IP subunit in Cu-deficient rat heart when compared with that of Cu-adequate rats. IMPACT: The elevation of fibulin-5, in particular, is important, because it implies that survival mechanisms have been initiated, which indirectly confirms that dietary copper deficiency leads to heart failure. [NP 107 Action Plan Component 4: Nutrient Requirements; ARS Strategic Plan Performance Measures 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new

classes of nutrients in the food supply and provide that information in databases.]

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

The usual routine transfer of nutritional knowledge about the nutritional, beneficial, and non-beneficial effects of trace elements was made through direct contact with industry representatives and the public and with other scientists through presentations at national and international meetings and professional publications.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

Jack T. Saari wrote an article which appeared in 2006 in the nutrition section of the Grand Forks Herald titled "Add Color to Your Diet with Fruits, Veggies."

W. Thomas Johnson wrote an article which appeared in September 2006 in the nutrition section of the Grand Forks Herald titled "DASH Away High Blood Pressure."

W. Thomas Johnson presented a talk in February 2006 at the Department of Anatomy and Cell Biology, University of North Dakota School of Medicine and Health Sciences titled "Maternal Copper Deficiency Has Negative Cardiac Effects in the First Generation."

W. Thomas Johnson presented a talk in August 2006 at the East Grand Forks Senior Citizen Center titled "DASH Away High Blood Pressure."

Scientific Publications: **Log 115:**

1. Johnson, W.T., Brown-Borg, H.M. 2006. Cardiac cytochrome-c oxidase deficiency occurs during late postnatal development in progeny of copper-deficient rats. Experimental Biology and Medicine. 231:172-180. 0000181570

2. Wang, J., Song, Y., Elsherif, L., Song, Z., Zhou, G., Prabhu, S.D., Saari, J.T., Cai, L. 2006. Cardiac metallothionein induction plays the major role in the prevention of diabetic cardiomyopathy by zinc supplementation. Circulation. 113:544-554. 0000177079

3. Relling, D.P., Esberg, L.B., Fang, C.X., Johnson, W.T., Murphy, E.J., Carlson, E.C., Saari, J.T., Ren, J. 2006. High-fat diet-induced juvenile obesity leads to cardiomyocyte dysfunction and upregulation of Foxo3a transcription factor independent of lipotoxicity and apoptosis. Journal of Hypertension. 24:549-561. 0000172125

4. Johnson, W.T., Newman, Jr., S.M. 2006. Cardiac mitochondrial function is altered in the adult offspring of copper-deficient dams [abstract]. Journal of Federation of American Societies for Experimental Biology. 20(5):A1065. 0000187381

5. Schuschke, D.A., Williams, C., Kang, Y.J., Saari, J.T. 2006. Cu-repletion promotes angiogenesis in the Cu-deficient rat heart [abstract]. Journal of Federation of American Societies for Experimental Biology. 20(4):A553. 0000187375

6. Saari, J.T., Reeves, P.G. 2006. Pinto beans are a good source of dietary copper [abstract]. Journal of Federation of American Societies for Experimental Biology. 20(4):A554. 0000187376

Project Number: 5450-51000-038-00D **Accession:** 0408766 **FY:** 2006

7. Zeng, H., Saari, J.T. 2006. New findings on protein expression on copper deficient rat heart with proteomic approach [abstract]. FASEB J. 20(4):A553. 0000187183

8. Zeng, H., Saari, J.T., Dahlen, G.M. 2005. Copper deficiency increases fibulin-5 (dance/evec) but decreases cytochrome c oxidase vib expression in rat heart. Inorganic Biochemistry. 100:186-91. 0000179965

9. Reeves, P.G., Saari, J.T. 2005. Bioavailability of copper from cooked dry beans [abstract]. Annals of Nutrition and Metabolism. 49(Suppl 1). p.2.3. 0000180163

10. Combs, G.F. 2006. Indications of magnesium and calcium deficiency in populations [abstract]. International Symposium on Health Aspects of Calcium and Magnesium in Drinking Water, Program and Abstracts, p. 41. 0000193356

11. Nielsen, F.H. 2006. Arsenic. In: Klasing, K.C., editor. Mineral Tolerance of Animals. 2nd Revised Edition. Washington DC; National Academies Press. p. 31-41. 0000168402

12. Johnson, W.T., Newman Jr, S.M. 2006. Hearts in adult offspring of copper-deficient dams exhibit decreased cytochrome c oxidase activity, increased mitochondrial hydrogen peroxide generation and enhanced formation of intracellular residual bodies. Journal of Nutritional Biochemistry. doi:10.1016/j.jnutbio.2006.03.005 0000190141

Approved: BLACKBURN WILBERT H **Date:** 09/26/2006

Project Number: 5450-51000-039-00D **Accession:** 0409965 **FY:** 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH **Principal Investigator:** CURTISS HUNT
Start Date: 08/01/2005 **Term Date:** 04/30/2009
National Programs: 107 N Human Nutrition
Title: MINERAL INTAKES FOR OPTIMAL BONE DEVELOPMENT AND HEALTH
Period Covered From: 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter?**

Osteoporosis is a major metabolic bone disease that affects at least 10 million Americans and costs over $14 billion annually in direct medical expenditures. The cause of this debilitating disease is known to be multifactorial with nutritional, genetic and hormonal components. It is recognized widely that manipulation of the diet to improve bone mineralization is the most cost-effective, practical approach in preventing osteoporosis. This approach calls for understanding all significant nutritional factors affecting bone mineral turnover. Such factors include, but are not limited to macronutrients (e.g. protein, lipids), minerals (e.g. calcium, magnesium, phosphorus, potassium, sodium, boron, copper, manganese, zinc), and vitamins (e.g., vitamins A, C, D, E, and K) which modulate such aspects of calcium homeostasis as intestinal absorption or renal excretion, hormone signals, and/or bone formation/bone resorption processes).

Calcium intakes of postmenopausal women in the US are considered to be inadequate, but the National Academy of Science, Food and Nutrition Board (FNB) was unable to set an Recommended Dietary Allowance (RDA) due to the paucity of pertinent calcium retention data. Thus, the FNB called for both short- and long-term studies to determine the limits of physiologic adaptation to changes in calcium intake and recognized the need for information concerning dietary factors that affect calcium absorption and/or retention. This project will address these needs by investigating the effects of dietary protein and prebiotics, and by following up on our recent finding that copper and zinc can function synergistically with calcium to prevent bone loss. The FNB also called for better information on the influences of dietary boron on calcium and vitamin D metabolism as these influences relate to bone health. We address this need through controlled studies of boron, specifically to determine unequivocally whether it is a dietary essential and, in that context, whether it affects the need for or utilization of calcium. Last, the FNB called for further research on magnesium, particularly the interrelationships of inadequate dietary magnesium intakes, indicators of magnesium status, and bone health outcomes including osteoporosis.
Animal and human experiments were conducted with the goal of establishing the calcium requirement and characterizing the modifying roles of animal proteins (in meat and milk), and zinc, copper, magnesium, and boron (as trace elements) on that requirement. Postmenopausal women and appropriate animal models (e.g., ovariectomized female rats) consumed varying amounts (low, adequate, and/or supranutritional) of calcium and the identified dietary factors while all other components of the diet remained constant. The response of the animals and humans to the dietary manipulations was ascertained by evaluating appropriate biochemical, physiological, and anatomical variables.

Project Number: 5450-51000-039-00D **Accession:** 0409965 **FY:** 2006

The research undertaken fell under National Program 107, Human Nutrition, and addressed goal 3.1.1 (Human Nutrition Requirements). The challenge of this component is to identify essential nutrients, determine their effects on reproduction, development, function and longevity, and to provide information that will be used to develop standards to optimize human health, well-being, and genetic potential throughout the life cycle. All priority objectives, especially mechanism of action, biomarkers, function and performance, and nutrient interactions apply to the research program. Outcomes of the research was knowledge that will facilitate establishment of an RDA for calcium with due consideration of the dietary factors that have significant potential to modify that requirement.

2. List by year the currently approved milestones (indicators of research progress)

Year 1 (FY 2005):
1. Enroll subjects and conduct study on estimating the Ca requirement by titration.

2. Conduct the first of a three year study on bone health and copper and zinc supplementation.

3. Complete in utero phase of boron essentiality study.

4. Initiate growth phase of three year study of boron essentiality.

Year 2 (FY 2006):
1. Complete data analyses from study on estimating the Ca requirement by titration.

2. Enroll subjects and conduct study on bone health during weight loss.

3. Conduct the second of a three year study on bone health and copper and zinc supplementation.

4. Report findings from in utero phase of boron essentiality study.

5. Conduct the second of a three year study of boron essentiality.

6. Conduct study on whether dietary boron affects calcium absorption.

7. Enroll subjects and conduct study on whether dietary inulin affects calcium absorption.

Year 3 (FY 2007):
1. Report on study on estimating the Ca requirement by titration.

2. Complete analyses from study on bone health during weight loss.

3. Conduct the third of a three year study on bone health and copper and zinc supplementation.

4. Conduct the third of a three year study of boron essentiality.

5. Conduct post-growth phase of boron essentiality study.

6. Enroll subjects and conduct study on whether magnesium status affects calcium retention and bone resorption.

7. Complete analyses from study on whether dietary inulin affects calcium

absorption.

Year 4 (FY 2008):

1. Report on study of whether bone health is affected by weight loss.

2. Complete analyses on study on bone health and copper and zinc supplementation.

3. Report findings from growth phase of boron essentiality study.

4. Report findings from post-growth phase of boron essentiality study.

5. Complete analyses on study whether magnesium status affects calcium retention and bone resorption.

6. Report findings from dietary inulin and calcium absorption study

3a. List the milestones (from the list in Question 2) that were scheduled to be addressed in FY 2006.

1. Complete data analyses from study on estimating the Ca requirement by titration; substitution of meta-analysis of existing human calcium balance data.

   Milestone Fully Met

2. Enroll subjects and conduct study on bone health during weight loss.

   Milestone Not Met

   Reason not met: Critical SY Vacancy

3. Conduct the second of a three year study on bone health and copper and zinc supplementation.

   Milestone Fully Met

4. Report findings from in utero phase of boron essentiality study.

   Milestone Substantially Met

5. Conduct the second of a three year study of boron essentiality.

   Milestone Fully Met

6. Conduct study on whether dietary boron affects calcium absorption.

   Milestone Fully Met

7. Enroll subjects and conduct study on whether dietary inulin affects calcium absorption.

   Milestone Not Met

   Reason not met: Critical SY Vacancy

3b. List the milestones (from the list in Question 2) that you expect to address over the next 3 years (FY2007, 2008 & 2009). What do you expect to accomplish, year by year, over the next three years under each milestone?

FY 2007:

Report on study on estimating the Ca requirement by titration.

A study to determine the amount of dietary calcium needed to maximize calcium

retention and minimize bone resorption in postmenopausal women was modified to determine the adult calcium requirement by performing a meta-analysis of existing human calcium balance data at the Center. Because existing experimental data are not adequate to assess the calcium requirement, the information from this study will be useful to Dietary Reference Committees in establishing a Recommended Daily Allowance for calcium in adults.

Complete analyses from study on bone health during weight loss.
A study to determine whether a balanced, high protein diet, one providing moderate amounts of carbohydrate, is adequate to maintain calcium homeostasis will not be completed because of a critical SY vacancy.

Conduct the third of a three year study on bone health and copper and zinc supplementation.
A three year study is underway to determine whether dietary supplements of copper and/or zinc enhance the ability of calcium to attenuate bone loss in postmenopausal women. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with zinc and copper, two elements that show special promise in affecting bone calcification.

Conduct the third of a three year study of boron essentiality during growth.
A three year study is underway to determine whether or not boron affects the quantitative need for calcium and is essential for bone health during the postnatal phase of development. Because adequate calcium intake continuous from development is considered critical for the formation and maintenance of a healthy skeleton, this research will characterize some of the interactions of calcium with boron, an element that benefits bone calcification.

Conduct post-growth phase of boron essentiality study.
A study will be completed on whether dietary boron affects the quantitative need for calcium and is essential for bone health during the post-growth phase of the life cycle. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with boron, an element that benefits bone calcification.

Enroll subjects and conduct study on whether magnesium status affects calcium retention and bone resorption.
A study planned to determine whether marginal magnesium status increases bone resorption and changes calcium needs in postmenopausal women was modified to determine the adult magnesium requirement by performing a cross-sectional statistical analyses of metabolic magnesium balance data collected at the Center over the last three decades. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, the information from this study will be useful to Dietary Reference Committees in establishing a revised Recommended Daily Allowance for magnesium, an element that may be a risk factor for osteoporosis.

Complete analyses from study on whether dietary inulin affects calcium absorption.
A study designed with healthy postmenopausal women to determine whether inulin, a prebiotic, sufficiently enhances calcium absorption to be of practical significance for bone health will not be completed because of a critical SY vacancy.

FY 2008:

Project Number: 5450-51000-039-00D Accession: 0409965 FY: 2006

Report on study of whether bone health is affected by weight loss.
A study to determine whether a balanced, high protein diet, one providing moderate amounts of carbohydrate, is adequate to maintain calcium homeostasis will not be completed because of a critical SY vacancy.

Complete analyses on study on bone health and copper and zinc supplementation
Data analysis will be completed from study to determine whether dietary supplements of copper and/or zinc enhance the ability of calcium to attenuate bone loss in postmenopausal women. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with zinc and copper, two elements that show special promise in affecting bone calcification. Information from this study will be useful to Dietary Reference Committees in establishing a Recommended Daily Allowance for calcium in postmenopausal women.

Report findings from growth phase of boron essentiality study.
Findings will be reported from a study on whether or not boron affects the quantitative need for calcium and is essential for bone health during the postnatal phase of development. Because adequate calcium intake continuous from development is considered critical for the formation and maintenance of a healthy skeleton, this research will characterize some of the interactions of calcium with boron, an element that benefits bone calcification. This information is needed by Dietary References Committees to help establish an adequate intake of boron in adolescents.

Report findings from post-growth phase of boron essentiality study.
Findings will be reported from a study on whether dietary boron affects the quantitative need for calcium and is essential for bone health during the post-growth phase of the life cycle. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with boron, an element that benefits bone calcification. This information is needed by Dietary References Committees to help establish an adequate intake of boron in mature adults.

Complete analyses on study whether magnesium status affects calcium retention and bone resorption.
A study planned to determine whether marginal magnesium status increases bone resorption and changes calcium needs in postmenopausal women was modified to determine the adult magnesium requirement by performing a cross-sectional statistical analyses of metabolic magnesium balance data collected at the Center over the last three decades. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, the information from this study will be useful to Dietary Reference Committees in establishing a revised Recommended Daily Allowance for magnesium, an element that may be a risk factor for osteoporosis.

Report findings from dietary inulin and calcium absorption study.
A study designed with healthy postmenopausal women to determine whether inulin, a prebiotic, sufficiently enhances calcium absorption to be of practical significance for bone health will not be completed because of a critical SY vacancy.

FY 2009:

Complete analyses on study on bone health and copper and zinc supplementation
Data analysis will be reported from study to determine whether dietary supplements of copper and/or zinc enhance the ability of calcium to attenuate bone loss in

postmenopausal women. Because osteoporosis is a multifactorial disease not prevented by adequate calcium and vitamin D nutrition alone, this research will characterize the interactions of calcium with zinc and copper, two elements that show special promise in affecting bone calcification. Information from this study will be useful to Dietary Reference Committees in establishing a Recommended Daily Allowance for calcium in postmenopausal women.

4a. List the single most significant research accomplishment during FY 2006.

New Estimation of the Magnesium Requirement for Men and Women:
Magnesium is an element that may be a risk factor for osteoporosis and current recommendations regarding the amount of magnesium needed to support health and optimal biological function are based on sparse balance data. ARS scientists at Grand Forks, ND, established a new estimation of the average magnesium requirement of healthy men and women by conducting secondary analyses of magnesium balance data generated in 27 human controlled feeding studies at the Grand Forks Human Nutrition Research Center over the last three decades. The findings suggest a magnesium requirement for healthy men and women (165 mg/d) that is lower than that estimated previously. This work will be very useful in setting the next Dietary Reference Intake for magnesium. This accomplishment was conducted under National Program 107 Human Nutrition; goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining human requirements for known classes of nutrients.

4b. List other significant research accomplishment(s), if any.

Boron Promotes Bone Healing After Tooth Extraction:
Slow or poor bone formation slows recovery time or healing after injury such as that which occurs with tooth extraction. A cooperative study between scientists at the Grand Forks Human Nutrition Research Center, University of Buenos Aires, and National Research Council of Argentina, showed that a diet low in boron affected bone healing after tooth extraction in mice and rats. Boron deprivation compared to usual intakes of boron resulted in markedly impaired alveolar bone healing after tooth extraction because of reduced bone formation (osteogenesis). This accomplishment is important because it shows that bone healing can be enhanced with a diet containing boron-rich foods (fruits, vegetables and pulses). This accomplishment was conducted under National Program 107 Human Nutrition; goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

Dietary Boron May Help Maintain Body Weight
Children that experience a rapid weight gain during infancy have been shown to be at greater risk for becoming obese during childhood. Dietary boron reduces body weight in adolescent rats while maintaining indicators of good health. ARS scientists at Grand Forks, ND conducted a study with pregnant rat dams to determine whether boron nutrition early in development is important for body weight of female offspring during late adolescence. They found that rat pups were lighter and had lower cholesterol and triglyceride levels in their blood if their dams consumed usual amounts of boron during pregnancy and lactation. This finding may be of importance in human nutrition where excess body weight is associated with a low intake of fruits and vegetables. These foods are good sources of dietary boron. This accomplishment was conducted under National Program 107 Human Nutrition; goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

Project Number: 5450-51000-039-00D Accession: 0409965 FY: 2006

Boron and Insulin Secretion
High levels of insulin in the blood over a long time cause the pancreas to become "exhausted" and to stop secreting insulin after awhile, a situation that results in diabetes. Scientists at the Grand Forks Human Nutrition Research Center, in cooperation with scientists at North Dakota State University, Fargo, ND, have shown that dietary boron reduced the amount of insulin in the blood by about 25% while maintaining the proper amount of glucose in male rats. This finding may be of importance in human nutrition where a decreased risk of developing a glucose tolerance abnormality is associated with frequent consumption of dairy products and fruits and vegetables and low alcohol intake. Fruits and vegetables are good sources of dietary boron. This accomplishment was conducted under National Program 107 Human Nutrition; goal 4 of IMPROVING THE NATION'S NUTRITION AND HEALTH; and part of performance standard 4.1.2 related to defining functions for emerging classes of nutrients.

4c. List significant activities that support special target populations.

None

4d. Progress report.

None

5. Describe the major accomplishments to date and their predicted or actual impact.

This report is the first for this CRIS project (certified in 2005), thus the accomplishments for the life of the project are incorporated in sections 4a and 4b.

6. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?

Information about the nutritional of beneficial aspects of ultratrace and trace elements as it became available was routinely transferred to a variety of customers. The customers included nutritional risk assessment groups through direct contact or organized meetings and workshops; the public through web pages of professional organizations, via the popular media; and other scientists through presentations at national and international meetings and professional publications.

7. List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).

Information was transferred to the public through a local public lecture and through the local newspaper (Grand Forks Herald) that was also placed on the Grand Forks Human Nutrition Research Center Home Page. Dr. Nielsen wrote an article entitled, "Nutrients Keeps Immune System in Balance." Dr. Hunt wrote an article entitled, Don't overlook importance of breast-feeding" and presented the lecture entitled "The bricks (calcium) or the mortar (everything else): which is more important in a healthy bone?" for the Spring 2006 Medical School for the Public seminar series, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND.

Scientific Publications: **Log 115:**

1. Combs, Jr., G.F., Hassan, N. 2005. The Chakaria food system study: household level, case-control study to identify risk factor for rickets in Bangladesh. European Journal of Clinical Nutrition. 59:1291-1301. 0000171569
2. Hunt, C., Butte, N.F., Johnson, L.K. 2005. Boron concentrations in milk from mothers of exclusively breast-fed healthy full-term infants are stable during 0000174589

the first four months of lactation. Journal of Nutrition. 135:2383-86.

3. Nielsen, F.H. 2006. A mild magnesium deprivation affects calcium excretion but not bone strength and shape, including changes induced by nickel deprivation, in the rat. Biological Trace Element Research. 110:133-149. 0000175182

4. Hunt, C. 2006. Dietary boron: evidence for essentiality and homeostatic control in humans and animals. In: Fangsen Xu, Heiner E. Goldback, Patrick H. Brown, Richard W. Bell, Toru Fujiwars, Curtiss D. Hunt, Sabine Goldberg, Lei Shi, editors. Advances in Plant and Animal Boron Nutrition. Boron 2005, September 10-13, 2005, Wuhan, China. p. 230-246. 0000188166

5. Nielsen, F.H., Stoecker, B.J., Penland, J.G. 2006. Boron as a dietary factor for bone microarchitecture and central nervous system function. In: Fangsen Xu, Heiner E. Goldback, Patrick H. Brown, Richard W. Bell, Toru Fujiwars, Curtiss D. Hunt, Sabine Goldberg, Lei Shi, editors. Advances in Plant and Animal Boron Nutrition. Boron 2005, September 10-13, 2005, Wuhan, China. p. 255-268. 0000185404

6. Nielsen, F.H. 2006. Silicon. In: Klasing, K.C., editor. Mineral Tolerance of Animals. 2ndd Revised Edition. Washington DC; National Academies Press. p. 348-356. 0000166583

7. Friel, J., Hunt, C. 2005. Boron and molybdenum content in infant formulas [abstract]. 12th International Symposium on Trace Elements in Man and Animals. p. 93. 0000177728

8. Hunt, C. 2005. Dietary boron: evidence for essentiality and homeostatic control in humans and animals [abstract]. Presented at Boron 2005, Wuhan, China, September 10-13, 2005. 0000182767

9. Hunt, C., Johnson, L.K. 2006. Estimation of magnesium requirements in men and women by cross-sectional statistical analyses of metabolic magnesium balance data [abstract]. FASEB J. 20(4):A182. 0000187239

10. Turner, K.K., Nielsen, B.D., O'Connor, C.I., Rosenstein, D.S., Schott, H., Womack, C.J., Nielsen, F.H., Orth, M.W. 2006. Sodium zeolite A supplementation to dairy calves [abstract]. Journal American Science. vol. 84, Suppl. 1/Journal Dairy Science. vol. 89, Suppl. 1. p.436-437. 0000192242

11. Nielsen, F.H., Stoecker, B.J., Penland, J.G. 2005. Boron as a dietary factor for bone microarchitechture and central nervous system function [abstract]. Abstract Book, The Third International Symposium on All Aspects of Plant and Animal Boron Nutrition, September 9-13, 2005, Huazhong Agricultural University, Wuhan, China, p 52. 0000182290

12. Nielsen, F.H. 2005. Effect of dietary silicon on bone turnover and the inflammatory response may be through an immune response involving osteopontin [abstract]. Journal of Dairy Science. 83(Suppl. 1):333. 0000179651

13. Nielsen, F.H., Stoecker, B. 2006. Dietary boron and fish oil have desirable effects on vertebra microarchitecture and strength [abstract]. FASEB J. 20(4):A561. 0000187229

14. Gorustovich, A.A., Steimetz, T., Nielsen, F.H., Guglielmotti, M.B. 2006. A histomorphometric study of alveolar bone healing in rats fed a boron-deficient diet [abstract]. FASEB J. 20(4):A24. 0000187245

15. Gorustovich, A.A., Steimetz, T., Nielsen, F.H., Guglielmotti, M.B. 2006. A histomorphometric study of alveolar bone modeling and remodeling in mice fed a boron-deficient diet [abstract]. FASEB J. 20(4):A195. 0000187252

16. Nielsen, F.H. 2006. Dietary magnesium deprivation confirmed by balance induces 0000193542

Project Number: 5450-51000-039-00D **Accession:** 0409965 **FY:** 2006

biochemical and functional changes including calcium balance in postmenopausal women [abstract]. International Symposium on Health Aspects of Calcium and Magnesium in Drinking Water, Program and Abstracts. p. 43-44.

Approved: BLACKBURN WILBERT H **Date:** 09/26/2006

Project Number: 5450-51000-039-05N Accession: 0407993 FY: 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Principal Investigator: FORREST H NIELSEN

Start Date: 03/01/2004 Term Date: 02/28/2009

National Programs: 107 N Human Nutrition

Title: HISTOMORPHOMETRIC AND BIOCHEMICAL ASSESSMENT OF THE POSSIBLE AUGMENTATION OF BONE HEALING AND REMODELING BY BORON

Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0038F

Organization Name: UNIVERSITY OF SALTA

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a non-funded cooperative agreement 58-5450-4-0038F between ARS and the National University of Salta, Argentina. Additional details of the research can be found in the report for the parent CRIS project 5450-51000-039-00D, Mineral Intakes for Optimal Bone and Joint Development and Health.

The purpose of this research is to determine whether boron is bioactive in bone formation and thus promotes bone growth and remodeling. Two experiments, one with mice and one with rats, showed that boron deprivation compared to nutritional intakes of boron resulted in impaired alveolar bone healing after tooth extraction because reduced osteogenesis (bone formation). The findings indicate that a low boron status results in suboptimal healing of bone after injury.

Scientific Publications:

Publications are reported in the parent CRIS 5450-510000-039-00D.

Scientific Publications: Log 115:

Approved: MCGUIRE MICHAEL R Date: 09/01/2006

Project Number: 5450-51000-039-06S **Accession:** 0408592 **FY:** 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH **Principal Investigator:** CURTISS HUNT
Start Date: 07/16/2004 **Term Date:** 09/30/2008

National Programs: 107 N Human Nutrition

Title: THE NUTRITIONAL ROLE OF BORON IN THE PREVENTION OF DIABETES

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0366

Organization Name: NORTH DAKOTA STATE UNIVERSITY

Progress and Outcomes:

4d. **Progress report.**

This report serves to document research conducted under specific cooperative agreement between ARS and North Dakota State University. Additional details of research can be in the report for the parent CRIS 5450-510000-039-00D,Mineral Intakes for Optimal Bone and Joint Development and Health.

To better define the mechanism by which dietary boron decreased plasma insulin levels and increased insulin sensitivity, we have successfully conducted a lab study to confirm our previous findings. The findings were summarized in the parent CRIS.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 09/26/2006

Project Number: 5450-51000-039-07T **Accession:** 0408848 **FY:** 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH **Principal Investigator:** FORREST H NIELSEN
Start Date: 07/01/2004 **Term Date:** 04/30/2008

National Programs: 107 N Human Nutrition

Title: EFFECT OF ARGININE SILICATE INOSITOL COMPLEX ON BONE AND JOINT HEALTH

Period Covered From: 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 04-5450-4-0415

Organization Name: NUTRITION 21, INC.

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a Trust Fund Cooperative Agreement between ARS and Nutrition 21, Inc., Purchase, NY. Additional details of research can be found in the report for the parent project 5450-51000-039-00D, Mineral Intakes for Optimal Bone and Joint Development and Health.

The purpose of this research is to determine whether silicon as a novel arginine silicate inositol complex or sodium metasilicate prevents any silicon deprivation enhancement of undesirable changes in markers of bone or connective tissue metabolism induced by pro-inflammatory agents. One experiment was performed to determine whether dietary silicon deprivation and supplementation alters the response to collagen-induced inflammatory arthritis, and any associated bone loss, in rats. The silicon supplementation did not alleviate the induced arthritis, nor did it markedly affect bone mineral composition. However, findings were obtained that indicated bone structure may be affected by silicon through altering the inflammataory or immune response. Silicon supplemented rats generally exhibited a more marked inflammatory response than silicon-deprived rats. The findings in the experiment suggested that physiological amounts of silicon promote the immune response, sex may influence the response to dietary silicon, and that both organic silicon complexes and inorganic silicon are effective in preventing the effects of silicon deprivation. The beneficial effect reported for silicon on bone health may be the result of the interaction of silicon between a cytokine and its cell surface receptor that activates intracellular signaling molecules. A second experiment was not successful in conclusively showing that dietary silicon changes the formation or structure of a glycosaminoglycan, particularly hyaluronan, such that its interaction with a cytokine that affects bone shape and strength was altered.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 09/06/2006

Project Number: 5450-51530-009-00D Accession: 0408299 FY: 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH Principal Investigator: HENRY C LUKASKI
Start Date: 04/03/2004 Term Date: 04/02/2009

National Programs: 107 N Human Nutrition

Title: MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? No
Terminate in Two Months? No

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter?

Suboptimal dietary intakes of essential micronutrients have been associated with chronic disorders such as obesity, diabetes, cardiovascular disease, depression and dementia. Further, national nutrition surveys indicate that dietary intakes of several essential minerals (e.g., calcium, copper, iron, magnesium, zinc) are less than recommended in many segments of the U.S. population and that mild-to-marginal deficiencies in these and other micronutrients are particularly likely in at-risk and underserved groups (e.g., women, children, elderly, minorities). Few controlled studies have been conducted of the relationships between micronutrients and chronic disease and the roles of such mediating factors as age, sex, body composition, special diets, lifestyle and genetic factors are poorly understood. For most micronutrients, the potential health benefits and mechanisms of action for physiological (healthy body weight and composition, energy metabolism, brain and cardiac function) and psychological (cognition, emotional and social adjustment, school/work performance) function have not been determined.
This project seeks to improve health and enhance quality of life by determining for healthy and at-risk groups micronutrient intakes that optimize physiological and psychological development, function and health. Specific project objectives are to: (1) develop new functional bases for establishing mineral element requirements; (2) identify mechanisms of action; and (3) determine the influence of mediating factors on mineral element requirements. This project is directly related to the following major components of the National Program Action Plan for Human Nutrition (107): Nutrient Requirements; Relationship between Diet, Genetics and Lifestyle and the Prevention of Chronic Disease; and Health-Promoting Interventions Strategies for Targeted Populations. The research addresses priority objectives including (1) determining functional markers of mineral intakes and status; (2) identifying mechanisms of action of mineral elements; (3) determining the influence of genetic, environmental and lifestyle factors on obesity and prevention of chronic disease; (5) identifying dietary intervention strategies effective with minority populations; (6) characterizing the role of mineral elements in achieving and maintaining optimal physiological and psychological development, function and health.

Controlled studies generate new knowledge to use in making recommendations for dietary intakes that promote optimal development, function and health throughout the life span. Dietary intakes and biochemical indices of mineral status are related to physiologic (e.g., healthy body weight and composition, physical fitness, energy metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine importance of specific minerals for optimal function and development. A mobile field

Project Number: 5450-51530-009-00D Accession: 0408299 FY: 2006

laboratory, community studies facilities, and a residential metabolic unit are used to conduct survey, supplementation, fortification, and controlled feeding studies with healthy and at-risk groups (e.g., school-aged children, rural elderly, minorities). Randomized controlled trials evaluate the effects of feeding graded dose amounts of minerals, such as iron, zinc, copper and magnesium, in the context of mediating factors (e.g., genotype, controlled stressors). Animal and cell culture studies enhance the efficacy of human studies and help determine the mechanisms of action of functional outcomes.

This project provides experimentally-derived information needed to establish recommendations for dietary intakes of mineral elements throughout the life span and to help American consumers choose foods that optimize physical and mental performance, social and emotional adjustment, and prevent or ameliorate chronic diseases such as obesity, diabetes, depression and dementia. Resulting information is also useful for evaluating food assistance programs, special diets, the efficacy of taking dietary supplements, and the potential benefits of value-added foods to maintain health and well-being. Primary customers for the products of this research are agricultural and commodity groups, the food industry, supplement manufacturers, policy makers, health and nutrition professionals and the general public.

2. List by year the currently approved milestones (indicators of research progress)

Year 1 (FY 2005)
1. Plan, obtain institutional and school board approvals, and initiate studies for participation of children and adolescents to determine relationships among zinc and iron nutrition and cognitive function, psycho-educational performance, body composition and growth.
2. Develop experimental protocol, initiate contacts with participating institutions, obtain institutional and administrative approvals, and initiate a cross-sectional epidemiologic study of nutrition, health and function in institutionalized and non-institutionalized elderly.
3. Plan and initiate study of the effects of dietary zinc and copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity.
4. Plan project and obtain approvals to initiate an observational study to determine the nutrient composition of foods in the food assistance programs and traditional foods on American Indian reservations.
5. Complete study to determine whether dietary boron restriction in rats influences eye development and behavioral changes induced by feeding diets high in omega-3 fatty acids and to relate any influence to a biochemical mechanism.

Year 2 (FY 2006)
1. Conduct study to determine in adolescents relationships between zinc and iron nutrition and cognitive function, psychoeducational performance, body composition, growth, and physical fitness at first site.
2. Plan project and initiate study to determine magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors.
3. Conduct study of relationships among zinc, copper and magnesium nutrition and physical and mental health of healthy elderly.
4. Analyze samples from study of effects of zinc and copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity; report results.
5. Plan project and initiate study of effects of different body composition phenotypes of rats on copper and zinc metabolism with increased physical activity in

rats.
6. Complete the nutrient composition survey of components of food assistance program and traditional American Indian foods.
7. Plan and initiate study of interaction of dietary boron and essential fatty acids in rats.
8. Establish communication, trust and collaborative partnerships with American Indian communities to begin discussions of health promotion, including obesity prevention.

Year 3 (FY 2007)
1. Conduct study of zinc supplementation of adolescents at second site.
2. Conduct study of magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors.
3. Plan and initiate study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity.
4. Develop nutrient database of commodity and traditional Native foods.
5. Initiate community focus groups to identify and report perceived health concerns/needs of tribal communities.
6. Analyze samples and data from observational study of the elderly; report results..
7. Plan experimental protocol for study of effects of graded zinc intake on adaptation to increased physical activity in humans.

Year 4 (FY 2008)
1. Conduct study of zinc supplementation of adolescents at third site.
2. Complete study of magnesium needs of postmenopausal women; analyze samples and data, and report results.
3. Develop experimental protocol, prepare and receive approval from host institution(s) and institutional review boards; initiate nutritional intervention study of the effects on mental and physical functions of the elderly.
4. Apply results of formative evaluation to development of culturally-appropriate interventions approaches, materials and programs for health promotion and obesity prevention in American Indian communities.
5. Complete the study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity.

3a. List the milestones (from the list in Question 2) that were scheduled to be addressed in FY 2006.

1. Conduct a study to determine in adolescents relationships between zinc and iron nutrition and cognitive function, psychoeducational performance, body composition, growth, and physical fitness at first site in North Dakota, Texas or American Indian Reservations.

   Milestone Not Met

   Reason not met: Redirection of research focus due to change
2. Plan project and initiate study to determine magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors.

   Milestone Not Met

   Reason not met: Redirection of research focus due to change

Project Number: 5450-51530-009-00D Accession: 0408299 FY: 2006

3a. 3. Conduct study of relationships among zinc, copper and magnesium nutrition and physical and mental health of healthy elderly.

Milestone Substantially Met

4. Analyze samples from study of effects of copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity; report results.

Milestone Substantially Met

5. Plan project and initiate study of effects of different body composition phenotypes of rats on copper and zinc metabolism with increased physical activity in rats.

Milestone Substantially Met

6. Complete the nutrient composition survey of components of food assistance program and traditional American Indian foods.

Milestone Fully Met

7. Plan and initiate study of interaction of dietary boron and essential fatty acids in rats.

Milestone Fully Met

8. Establish communication, trust and collaborative partnerships with American Indian communities to begin discussions of health promotion, including obesity prevention.

Milestone Substantially Met

3b. **List the milestones (from the list in Question 2) that you expect to address over the next 3 years (FY2007, 2008 & 2009). What do you expect to accomplish, year by year, over the next three years under each milestone?**

FY 2007
1. Milestone: Conduct study of zinc supplementation of adolescents at second site. Anticipated outcome: determine the effects of zinc supplementation at recommended and higher amounts on growth, development, body composition, cognition, social adaptation, and physical fitness of youth.

Because of a shift in research focus due to a change in priorities, this milestone will not be completed. The addition of a new SY to this CWU enables the addition of a new milestone. Milestone: Plan and initiate a local/regional survey to determine barriers and facilitators to healthful eating and physical activity in American Indians. Anticipated Accomplishment: Findings will be used to facilitate planning of effectiveness study as part of the ARS Obesity Prevention Initiative.

2. Milestone: Conduct study of magnesium requirements of postmenopausal women with outcomes of magnesium nutritional markers, inflammatory responses and cardiovascular risk factors. Anticipated outcomes: provide needed evidence for recommendation of magnesium intake in a population group for whom no objectives are available, determine effects of magnesium intakes commonly consumed by older women on risk factors for cardiovascular disease and bone loss, and validate new measures of magnesium status.

Because of a shift in research focus due to a change in priorities, this milestone will not be completed. However, the following milestone will be added. Milestone: Plan and initiate a contingency study using experimental animals to determine whether a diet high in n-6 fatty acids, compared to a diet high in n-3 fatty acids,

exacerbates a marginal magnesium deficiency resulting in increased pro-inflammatory neuropeptides and oxidative stress detrimental to cardiovascular function and neurological development. Anticipated Accomplishment: Dietary fatty acids that enhance oxidative stress and the release of pro-inflammatory cytokines and prostaglandins exacerbate marginal magnesium deficiency and increased risk of cardiovascular dysfunction.

3. Milestone: Plan and initiate study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity. Anticipated Accomplishment: Determine the effects of different levels of physical activity on copper nutritional status and mechanisms of metabolic adaptation to increased physical activity.

4. Milestone: Develop nutrient data base of commodity and traditional Native foods. Anticipated Accomplishment: Provide a needed and practical nutrient data base for use by American Indian and other health professionals to assist in planning healthful diets required in the promotion of healthy body weight.

5. Milestone: Initiate community focus groups to identify and report perceived health concerns/needs of tribal communities. Anticipated Accomplishment: Develop a culturally-appropriate and sustainable program for health body weight in American Indian communities.

6. Milestone: Analyze samples and data from observational study of the elderly; report results. Anticipated outcomes: determine effects of supplementation of limiting nutrients (e.g., zinc, magnesium, vitamin B12, etc.) on quality of life measures of the elderly.

Because of a critical SY vacancy, this milestone will not be completed.

7. Plan experimental protocol for study of effects of graded zinc intake on adaptation to increased physical activity in humans. Anticipated outcomes: determine if dietary zinc when fed in an amount consistent with usual intakes of US adults, compared to the recommended intake, results in impaired adaptation to increased physical activity at a level proposed in the Dietary Guidelines for Americans.

Because of a shift in research focus due to a change in priorities, this milestone will not be completed. Milestone: Plan multi-center ARS Efficacy Trial to evaluate the Dietary Guidelines for Americans to attain and maintain healthy body weight in adults. Findings will be used to facilitate planning of an effectiveness study as part of the ARS Obesity Prevention Initiative.

FY 2008
1. Milestone: Conduct study of zinc supplementation of adolescents at third site. Anticipated result: determine the effects of zinc supplementation at recommended and higher amounts on growth, development, cognition, social adaptation, and fitness of youth; contribute information needed to develop zinc requirement of adolescents.

Because of a shift in research focus due to a change in priorities, this milestone will not be completed. The addition of a new SY to this CWU enables the addition of a new milestone. Milestone: Complete local/regional survey to determine barriers and facilitators to healthful eating and physical activity in American Indians. Anticipated Accomplishment: Findings will be used to facilitate planning of effectiveness study as part of the ARS Obesity Prevention Initiative.

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2006

2. Milestone: Complete study of magnesium requirements for optimal cardiovascular, neurological and psychological function in postmenopausal women; analyze samples and data, and report results. Anticipated outcome: provide evidence to Food and Nutrition Board for use in establishing recommendations for magnesium intakes for postmenopausal women to optimize health and valid biochemical indicators of human magnesium status for use in national nutritional surveys.

Because of a shift in research focus due to a change in priorities, this milestone will not be completed. However, the following milestone will be added. Milestone: Complete and report findings from the study of the impact of dietary fatty acid composition on the severity of marginal magnesium deficiency in experimental animals. Anticipated Accomplishment: Analyses will show the dietary fatty acids that enhance oxidative stress and the release of pro-inflammatory cytokines and prostaglandins exacerbate marginal magnesium deficiency such that it impairs neurological development and motor function.

3. Milestone: Develop experimental protocol, prepare and receive approval from host institution(s) and institutional review boards; initiate nutritional intervention study of the effects on mental and physical functions of the elderly. Anticipated outcomes: determine effects of supplementation of limiting nutrients (e.g., zinc, magnesium, vitamin B12, etc.) on quality of life measures of the elderly.

Because of a critical SY vacancy, this milestone will not be completed. However the following milestone will be added. Initiate local/regional study to determine the efficacy of the diet and physical activity guidelines to facilitate healthy weight in overweight initially under controlled feeding and exercise environments and then in free-living conditions people (ARS Efficacy Trial). Anticipated Accomplishment: Findings will be used to facilitate planning of effectiveness study as part of the ARS Obesity Prevention Initiative.

4. Milestone: Apply results of formative evaluation to development of culturally-appropriate interventions approaches, materials and programs for health promotion and obesity prevention in American Indian communities. Anticipated Accomplishment: Develop a culturally-appropriate and sustainable program for health body weight in American Indian communities.

5. Milestone: Complete exercise training study; analyze samples; report results. Anticipated Accomplishment: Provide evidence of any effects of increased physical activity on copper nutritional status and physiological adaptations to aerobic exercise training.

FY 2009
1. Milestone: Based on findings from survey and efficacy studies conducted at this and other ARS research centers during FY 2007 and FY 2008, plan and initiate a study to determine the feasibility and effectiveness of following the Dietary Guidelines for Americans in local/regional communities, particularly rural and American Indian communities. Anticipated Accomplishment: Findings will establish the practical utility of following the current Dietary Guidelines for Americans as a means to achieve and maintain a healthy weight at all ages and in all peoples; thus, findings will directly address the ARS Obesity Prevention Initiative.

4a. List the single most significant research accomplishment during FY 2006.

Nutrient Composition of Plant Foods of Northern Plains Indians

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2006

Determination of the nutrient composition of traditional American Indian plant foods was completed and shared with leaders of the participating tribes; this information will be included in a new data released by USDA, ARS. This analysis was done in response to requests of three tribes in North Dakota and to contribute to a national nutrient database of foods from American Indians and Alaska Natives. After receiving permission from tribal governing councils, collaborators from United Tribes Technical College (Bismarck, ND) and representatives from each tribe collected Native plant foods in a culturally respectful and sensitive manner. Samples were received by the ARS Nutrient Data Laboratory (Beltsville Human Nutrition Research Center), processed and sent for analysis to contract laboratories. Knowledge of the nutrient content of traditional plant foods is important in planning and implementing culturally-important interventions to prevent obesity, diabetes and cardiovascular disease that are present among American Indians at rates much greater than among non-Indian populations in the area. This work is related to National Program 107, Nutrient Requirements: Relationships between Diet, Genetics, and Lifestyle and Prevention of Chronic Disease and Health-Promoting Intervention Strategies for Targeted Populations.

4b. List other significant research accomplishment(s), if any.

ARS National Obesity Prevention Initiative
Three scientists from this unit, in collaboration with scientists from other ARS Centers, developed two multi-center, multi-year research plans to address obesity prevention in the US. Rates of overweight and obesity are increasing despite efforts by public health organizations. The Departments of Health and Human Services and Agriculture have disseminated the Dietary Guidelines for Americans which provides guidelines for diet and physical activity to promote healthy body weight through the life span. One project will use surveys to determine barriers and facilitators to healthful eating and physical activity in all segments of the US population. The second study will determine the efficacy of the diet and physical activity guidelines to facilitate healthy weight in overweight people initially under controlled feeding and exercise environments and then in free-living conditions. Results of these studies will be used to implement another multi-center, multi-year investigation to assess the effectiveness of the Dietary Guidelines in the American public. This work is related to National Program 107, Nutrient Requirements: Relationships between Diet, Genetics, and Lifestyle and Prevention of Chronic Disease and Health-Promoting Intervention Strategies for Targeted Populations.

Micronutrient Nutrition and Memory Function in Older Adults
We investigated the effect of age on memory function (short- and long-term prose recall) mediated by micronutrient intakes and/or status, and found an inverse association between memory function and B12, magnesium and zinc intakes and/or status in healthy, free-living adults in both age groups. Findings indicate that, while micronutrient nutrition may be important for cognitive function in general and memory function in particular, it does not significantly affect age-related differences in memory function. This work relates to the ARS Strategic Plan to Improve the Nation's Nutrition and Health (Goal 4 of National Program 107 - Human Nutrition) and Objective 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients.

National Policies, Regulations and Guidelines
Three scientists in this CWU served on National Academies of Science, Institute of Medicine panels and participated in IOM workshops. These panels and workshops issued reports that serve as a scientific knowledge base used to formulate national

policies and regulations, and to generate dietary and physical activity guidelines, such as the Dietary Reference Intakes, Dietary Guidelines for Americans, Mineral Requirements for Animals, Military Dietary Reference Intakes, and Rations for Combat Operations. This group contributed as authors to 9 National Academies of Science publications this past year, and as members to the interpretations and recommendations of the panels as a whole.

4c. List significant activities that support special target populations.

Scientists in the unit continue to work with American Indians to develop successful partnerships and to promote research on health promotion. Examples include participating in the 5th Annual American Indian Research Forum as part of the University of North Dakota's Indian Association Timeout Celebration, where current community efforts underway to promote improved health and major research needs were identified and discussed, and initiating a Cultural Awareness Workshop at United Tribes Community College, attended by the researchers, technicians and administrators from throughout the Northern Plains Area. These activities directly support Grand Forks Human Nutrition Research Center programs to improve the nutrition and health of this at-risk and underserved population in our region, and facilitate accomplishment of the milestone related to health promotion and obesity prevention in American Indian communities.

In addition, two important Specific Cooperative Agreements were completed to promote collaborative research partnerships with Cankdeska Cikana Community College (Spirit Lake Reservation) and University of North Dakota. These agreements seek to formalize relationships to initiate discussion geared to develop culturally-appropriate activities and intervention to promote health and prevent obesity and diabetes among American Indians in the Northern Great Plains. One project scheduled to begin this fall will use qualitative methods to identify and characterize barriers and facilitators associated with potential nutrition and lifestyle changes to achieve and maintain a healthy body weight and reduce risk of chronic diseases and mitigate disease symptoms. This work is related to National Program 107, Nutrient Requirements: Relationships between Diet, Genetics, and Lifestyle and Prevention of Chronic Disease and Health-Promoting Intervention Strategies for Targeted Populations.

5. Describe the major accomplishments to date and their predicted or actual impact.

Copper Requirement for Men
We found increased losses of copper and increased energy needs during low-level physical activity in parallel with decreased activity of an energy-regulating copper-containing enzyme in muscle when 0.9 compared to 1.6 mg of copper was fed. These findings reveal that moderate copper intake adversely impacts the ability to perform work and relates this problem to the reduced activity of an important copper protein that regulates cellular energy production. This information will be useful in making recommendations on nutrient intakes to promote health and well-being of the public, as they suggest that the current RDA for copper may be insufficient to promote optimal function. This accomplishment falls within the ARS Strategic Plan to Improve the Nation's Nutrition and Health (Goal 4 of National Program 107 - Human Nutrition) and Objective 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients.

Research Partnerships with American Indian Communities
A major accomplishment is the development of trust and respect among the American Indian tribes in North Dakota that has resulted in the completion of a survey of

Native health, nutrition and physical activity, and in research agreements to analyze traditional Native foods and conduct focus group studies in Native communities to determine barriers and facilitators associated with adherence to the Dietary Guidelines for Americans. ARS and tribal partners now jointly develop projects to the benefit of both parties. This accomplishment falls within the USDA, ARS Strategic Plan to Improve the Nation's Nutrition and Health (Goal 4 of National Program 107 - Human Nutrition) and Objective 4.1.3: Determine food consumption patterns of Americans, including those of different ages, ethnicity, regions, and income levels. Provide sound scientific analyses of the U.S. food consumption information to enhance the effectiveness and management of the Nation's domestic food and nutrition assistance programs. It also addresses Action Plan component of Health Promoting Strategies for Targeted Populations and focuses on this at-risk and underserved population in our region; it also facilitates accomplishment of the milestone related to health promotion and obesity prevention in American Indian communities.

Beneficial Effects of Fish Oil on Vision are Most Evident when Boron Status is Low
The beneficial effects of fish oil, which is high in long chain n-3 polyunsaturated fatty acids (n-3 PUFA), on vision was more marked in rats with a restricted intake of dietary boron, and the beneficial effects of boron on vision was more evident in rats fed a diet high in n-6 PUFA. Some studies show that, compared to diets high in omega-6 PUFA, diets high in long chain n-3 PUFA enhance sensory functions, cognitive functions and brain development, but other studies do not. The inconsistency may be the result of different intakes of another nutrient, such as boron, shown to affect similar functions as long chain n-3 PUFA. The beneficial effects of fish oil or long-chain n-3 PUFA supplementation may be most evident when dietary boron restriction, combined with high omega-6 PUFA intake, has impaired behavioral and vision functions. This accomplishment falls within the ARS Strategic Plan to Improve the Nation's Nutrition and Health (Goal 4 of National Program 107 - Human Nutrition) and Objective 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients.

Muscle Cytochrome c Oxidase Activity as a Marker of Copper Status
Identification of the specificity of muscle cytochrome c oxidase activity as a marker of copper nutritional status and its potential for future research to determine the interaction of dietary copper restriction and supplementation on adaptation of muscle metabolism to increased physical activity. This work has lead to a study in men which confirmed that the activity of the enzyme is decreased when dietary copper is low and results in decreased copper nutritional status. This accomplishment falls within the ARS Strategic Plan to Improve the Nation's Nutrition and Health (Goal 4 of National Program 107 - Human Nutrition) and Objective 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients. It also addresses as the milestone (FY 2005) to relate dietary copper and physical activity.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

There are no CRADAs, licenses or patents to report.

Transfer of technical information to other scientists and customers occurred through

presentations at national and international meetings, conferences sponsored by commodity groups, professional publications and service on national advisory committees. Knowledge about the health benefits of mineral nutrients was transferred by routine contacts to representatives of industry and policy-making and regulatory federal agencies. Transfer of knowledge to the public occurred through contacts with media representatives and by direct contacts with the public.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

Popular Press
Colby, S.E. Our Health, Our Choices. Red Nation News, November 2005.

Colby, S.E. Building Healthy Communities. Grand Forks Herald, January 2006.

Lukaski, H.C. Adequate Copper Saves Bones During Weight Loss. Agricultural Research, March 2006, p. 22.

Lukaski, H.C. Low Zinc Levels Could Take Your Breath Away. Agricultural Research, April 2006, p. 18.

Penland, J.G. Improve Mental Health with Good Food, Exercise. Grand Forks Herald, July 2006, p. D-1.

Media Coverage
S. Colby was interviewed for stories in the Grand Forks Herald, Red Nation News, and Red River Valley Women Today.

H. Lukaski described research findings on the roles of copper, magnesium and zinc in promoting health and various aspects of physical function and performance that were published in stories in Rodale Press, as well as magazines including Allure, Daily Health News, Eating Well, Family Circle, Input Fitness, Real Simple, Science News, SELF and Women's Health magazines.

J. Penland was interviewed/cited in several stories on minerals and mental function and mood states, appearing in publications such as Child, Grand Forks Herald, Rodale Press, SELF, and First for Women.

Presentations
Scientists in the unit made 20 presentations to industry, governmental and educational entities, 6 of which occurred during the 4th Quarter of FY-05, and 14 during the 1st-3rd Quarters of FY-06. The most important presentations included:

Nielsen, F.H. The Toxicity of Minerals That May Be Advocated for Animal Health and Production through Reasons Other Than Nutritional Need. Joint Meeting of the American Dairy Science Association, American Society for Animal Science and Canadian Society for Animal Science, Cincinnati, OH, July, 2005.

Gray, J.S., Penland, J.G., Lambert, P., Gonzalez, J. Northern Plains Indians: cultural identity and lifestyle factors in depression. American Psychological Association, Washington DC, August 2005.

Nielsen, F.H., Stoecker, B.J., Penland, J.G. Boron as a dietary factor for bone microarchitecture and central nervous system function. Boron 2005, Wuhan, China, September, 2005.

Penland, J.G. Nutrition and Memory in Aging. Golden Pioneers, Thief River Falls, MN, September, 2005.

Lukaski, H.C. The Grand Forks Human Nutrition Research Center - What It Does for You. East Grand Forks Senior Center, East Grand Forks, MN, September 13, 2005.

Lukaski, H.C. Quackery in Marketing Dietary Supplements. Camp Diabetes 2005, Maplelake on the White Earth Reservation, Calloway, MN, September 15, 2005.

Penland JG. Zinc and Behavior in School-Aged Children. North Dakota State University Fall Extension Conference, Fargo, ND, October, 2005.

Lukaski, H.C. Health-Promoting Qualities of Regionally Produced Agricultural Commodities. 23rd Annual Harvest of Knowledge, Agri-Women's Conference, Grand Forks, ND, October 28, 2005.

Colby, S.E. Hunger: Our World, Our Nation, Our Home. The University of North Dakota Hunger Banquet, Grand Forks, ND, November 15, 2005

Colby, S.E. Dietary Acculturation of Mexican American Immigrants: A Case Study, American Public Health Association, Philadelphia, PA, December 13, 2005.

Gray, J.S., Knudson, A.D., Penland, J.G. Impact of Food Insecurity on Depression Among Northern Plains Indians. American Public Health Association, Philadelphia, PA, December, 2005.

Lukaski, H.C. Fitness or Fatness as a Predictor of Health. Annual Meeting of the Fargo-Moorhead Dietetic Association, Fargo, ND, January 21, 2006.

Lukaski, H.C. Increased Physical Activity - Key to Healthy Body Weight and Composition. Grand Forks Public Schools Wellness Program, Grand Forks, ND, January 31, 2006.

Colby, S.E. Registered Dietitians in Research. University of North Dakota, Grand Forks, ND, March 1, 2006.

Colby, S.E. Targeting Learning Styles: A bridge to effective behavior change. North Dakota Nutrition Council, Bismarck, ND, March 23, 2006.

Gray, J.S., Penland, J.G., Knudson, A.D. Health, lifestyle, food insecurity and acculturation factors related to depression in Northern Plains Indians. National Rural Health Association, Reno, NV, May, 2006.

Colby, S.E. Traditional Diet and Lifestyles: Perspectives from Mexico. Society for Nutrition Education, San Francisco, CA, July 17, 2006.

Lukaski, H.C. Body Composition - What It Should Mean to You. East Grand Forks Senior Center, East Grand Forks, MN, March 21, 2006.

Lukaski, H.C. Dietary and Sports Supplements - What They Are and Do They Work? Minnesota Soccer Coaches Certification Conference, Moorhead, MN, June 20, 2006.

Lukaski, H.C. Body Composition - What About It Predicts Chronic Disease in Humans? Rotary Club, Grand Forks, ND, July 11, 2006.

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2006

Scientific Publications: **Log 115:**

1. Colby, S.E. 2006. Nutrition marketing and the worldwide obesity epidemic [abstract]. Proceedings of the Allied Academies International Conference. New Orleans, LA. April 12-15. p.7. 0000193697
2. Greger, J.L., Nielsen, F.H., Klasing, K.C. 2005. Potential adverse effects on humans consuming excess minerals in animal products [abstract]. Journal Dairy Science. 88(Suppl. 1):333. Presented at ADSA-ASAS-CSAS 2005 Joint Meeting, Cincinnati, OH, July 24-28, 2005. 0000178142
3. Klein, C.J., Nielsen, F.H., Moser-Veillon, P.B. 2006. Trace element loss following trauma and during continuous renal replacement therapy [abstract]. Journal of Parenteral and Enteral Nutrition. 30(2):536-7. 0000186432
4. Lukaski, H.C. 2006. Zinc (Chapter 15). In: Driskell, J.A., Wolinsky, I (eds). Sports Nutrition, Vitamins and Trace Elements. New York, CRC Press. p. 217-234. 0000171092
5. Lukaski, H.C. 2006. Protein turnover and mineral metabolism. In: Institute of Medicine of the National Academies, editors. Mineral Requirements for Military Personnel: Levels Needed for Cognitive and Physical Performance During Garrison Training. Washington, DC:National Academies Press. p.338-343. 0000183171
6. Lukaski, H.C. 2006. Zinc, magnesium, and copper requirements and exercise. In: Institute of Medicine of the National Academies, editors. Mineral Requirements for Military Personnel: Levels Needed for Cognitive and Physical Performance During Garrison Training. Washington, DC: National Academies Press. p. 436-451. 0000183172
7. Lukaski, H.C., Penland, J.G. 2006. No effect of chromium picolinate supplementation on body weight/composition of women fed controlled diets [abstract]. Medicine and Science in Sports and Exercise. 38(5):S126. 0000187095
8. Nielsen, F.H. 2006. Boron. In: Klasing, K.C., editor. Mineral Tolerance of Animals. 2nd Revised Edition. Washington DC; National Academies Press. p. 60-67. 0000168407
9. Nielsen, F.H. 2006. Nickel. In: Klasing, K.C., editor. Mineral Tolerance of Animals. 2nd Revised Edition. Washington DC; National Academies Press. p. 276-283. 0000166589
10. Nielsen, F.H. 2006. Other minerals. In: Klasing, K.C., editor. Mineral Tolerance of Animals. 2nd Revised Edition. Washington DC; National Academies Press. p. 428-448. 0000174603
11. Nielsen, F.H. 2006. Boron, manganese, molybdenum, nickel, silicon, and vanadium. In: Driskell, J.A., Wolinsky, I., editors. Sports Nutrition Vitamins and Trace Elements. 2nd edition. Boca Raton, FL: CRC Press. p. 287-320. 0000176038
12. Nielsen, F.H. 2006. Chapter 41, the ultratrace elements. In: Stipanuk, M.H., editor. Biochemical, Physiolgical, & Molecular Aspects of Human Nutrition. St. Louis, MO: Saunders Elsevier. p. 1143-1163. 0000174912
13. Nielsen, F.H. 2005. The toxicity of minerals that may be advocated for animal health and production through reasons other than nutritional need [abstract]. Journal of Dairy Science. 88(Suppl. 1):333. Presented at ADSA-ASAS-CSAS 2005 Joint Meeting in Cincinnati, OH, July 24-28, 2005. 0000176911
14. Nielsen, F.H., Penland, J.G. 2006. Boron deprivation alters rat behaviour and brain mineral composition differently when fish oil instead of safflower oil is the diet fat source. Nutritional Neuroscience. 9(1-2):105-112. 0000190788
15. Nielsen, F.H., Penland, J.G. 2006. Dietary boron, fish oil, and their 0000187240

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2006

interaction affect rat behavior and brain mineral composition [abstract]. FASEB J. 20(4):A176.

16. Penland, J.G. 2006. Zinc and other mineral nutrients required for cognitive function and behavior in military personnel. In: Institute of Medicine (editor), Mineral Requirements for Military Personnel: Levels Needed for Cognitive and Physical Performance During Garrison Training. Washington, DC,National Academies Press. p. 420-436. 0000183310

17. Penland, J.G., Finley, J.W., Gao, J. 2006. Selenium status is associated with mood states and cognitive function in Chinese men [abstract]. Journal of Federation of American Societies for Experimental Biology. 20(5):A1070. 0000187383

18. Shafer, I., Lohse, B. 2006. How to conduct a cognitive interview: A nutrition education example. (http://www.csrees.usda.gov/nea/food/pdfs/cog_interview.pdf) 0000185067

19. Swain, J.H., Penland, J.G., Johnson, L.K., Hunt, J.R. 2006. Energy, mood and attention did not consistently improve with iron status in non-anemic women with moderate to low iron stores [abstract]. FASEB J. 20(4):A191. 0000187202

Approved: BLACKBURN WILBERT H **Date:** 09/26/2006

Project Number: 5450-51530-009-01T Accession: 0406520 FY: 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH

NPL Leader: MARY J KRETSCH Principal Investigator: HENRY C LUKASKI

Start Date: 10/01/2002 Term Date: 09/30/2007

National Programs: 107 N Human Nutrition

Title: DETERMINATION OF THE EFFECTS OF BUCKWHEAT ON MANAGEMENT OF PRE-DIABETES AND NON-INSULIN DEPENDENT DIABETES (NIDDM) IN ANIMAL MODELS

Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0402

Organization Name: MINN-DAK GROWERS, LTD.

Progress and Outcomes:

4a. List the single most significant research accomplishment during FY 2006.

None

4b. List other significant research accomplishment(s), if any.

None

4c. List significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under a trust fund cooperative agreement between ARS and Minn-Dak Growers, Ltd. Additional details of research can be found in the report for the parent project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

We had planned a trial to evaluate the effect of a commercial extract of buckwheat, including fagopyritols, on insulin sensitivity of male, Zucker obese (ob/ob). Our collaborator was unable to provide the extract with adequate concentrations of a mixture of fagopyritols to undertake the study. Until this extract is available, the study will not proceed.

Scientific Publications: Log 115:

Approved: MCGUIRE MICHAEL R Date: 09/25/2006

Project Number: 5450-51530-009-02N Accession: 0408500 FY: 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH Principal Investigator: JAMES G PENLAND
Start Date: 10/18/2004 Term Date: 09/30/2007
National Programs: 107 N Human Nutrition
Title: NUTRITION EFFECTS ON COGNITIVE PERFORMANCE OF YOUNG VERSUS ELDERLY COMMUNITY-LIVING ADULTS
Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? No
Terminate in Two Months? No
Agreement Number: 58-5450-5-0101N
Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

4a. List the single most significant research accomplishment during FY 2006.

None

4b. List other significant research accomplishment(s), if any.

None

4c. List significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under non-funded cooperative agreement #58-5450-5-101N between the ARS and the University of North Dakota Psychology Department. Additional information can be found in the report for the parent project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

Older adults demonstrate poorer memory function that younger adults, even in the absence of chronic disease. Older adults also frequently consume less than recommended intakes of several micronutrients, including vitamin B12, folate, magnesium, zinc and copper. Because micronutrient nutrition has been related to cognitive function in several age groups, we investigated whether age-related differences in memory function (short- and long-term prose recall) are mediated by micronutrient intakes and/or status, and found an inverse association between memory function and B12, magnesium and zinc intakes and/or status in healty, free-living adults in both age groups. Findings indicate that while micronutrient nutrition may be important for cognitive function in general and memory function in particular, it does not appear to significantly mediate age-related differences in memory function. Future studies must determine whether micronutrients are important for memory in older adults with chronic disease and for other cognitive functions. This work relates to the ARS Strategic Plan to Improve the Nation's Nutrition and Health (Goal 4 of National Program 107 - Human Nutrition) and Objective 4.1.2: Define functions, bioavailability, interactions, and human requirements (including effects such as genetic, health status, and environmental factors) for known, emerging, and new classes of nutrients.

Scientific Publications: Log 115:

Project Number: 5450-51530-009-02N **Accession:** 0408500 **FY:** 2006

Approved: MCGUIRE MICHAEL R **Date:** 09/01/2006

Project Number: 5450-51530-009-03N **Accession:** 0409328 **FY:** 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH **Principal Investigator:** HENRY C LUKASKI
Start Date: 05/26/2005 **Term Date:** 03/31/2007

National Programs: 107 N Human Nutrition

Title: ASSESSMENT OF MINERAL LOSSES IN SWEAT DURING PHYSICAL ACTIVITY

Period Covered From: 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-5-0107N

Organization Name: GATORADE SPORTS SCIENCE INSTIT.

Progress and Outcomes:

4a. List the single most significant research accomplishment during FY 2006.

Sweat Mineral Losses in Youth During Controlled Exercise
We performed the first study to evaluate mineral concentrations from specific sites on the bodies of youth and adolescent athletes during physical activity. The principal finding was that sweat mineral concentrations differed by sex; mineral concentrations were greater in males than females. Collaborators from Gatorade Sports Science Institute (Barrington, IL) recruit members of elite youth soccer teams to wear unobtrusive sweat collection patches during training sessions. This preliminary information is important in planning future studies to assess the impact of surface mineral losses on mineral nutritional status of humans. This work is related to Human Nutrition Action Plan 107 and addresses the area of Nutrient Requirements.

4b. List other significant research accomplishment(s), if any.

None

4c. List significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under non-funded cooperative agreement #58-5450-5-107N between ARS and Gatorade Sport Science Institute. Additional information can be found in the report for the parent project 5450-51530-009-00D Micronutrient Roles in Physiology and Health.

Another study is underway to develop a standard reference material for use in analysis of human sweat samples. The only available reference material with a similar matrix as sweat is an NIST water standard. However, the mineral concentrations are not in the same ranges as those found in human sweat. Thus, there is a critical need to develop this standard for future research.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/01/2006

Project Number: 5450-51530-009-04N Accession: 0410137 FY: 2006
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: MARY J KRETSCH Principal Investigator: GERALD F COMBS
Start Date: 03/09/2006 Term Date: 09/30/2010

National Programs: 107 N Human Nutrition

Title: MINERAL NUTRITION RESEARCH

Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? No
Terminate in Two Months? No

Agreement Number: 58-5450-6-0101N

Organization Name: US ARMY RES INST ENVIR MEDICINE

Progress and Outcomes:

4a. List the single most significant research accomplishment during FY 2006.

None

4b. List other significant research accomplishment(s), if any.

None

4c. List significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under a non-funded cooperative agreement between ARS and the United States Army Research Institute for Environmental Medicine (USARIEM). Additional details of research can be found in the report for the parent project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

The project represents the commitment of the Grand Forks Human Nutrition Research Center and the United States Army Research Institute for Environmental Medicine (USARIEM) to collaborate in studies of mutual value in the general area of mineral nutrition. The focus of efforts this year were in the development of methods for collecting and analyzing human sweat losses. Methodological development was initiated by the Grand Forks Human Nutrition Research Center to analyze the micronutrient contents of human sweat samples, with particular attention to zinc, copper, iron and calcium, magnesium, sodium, potassium and chloride. Collaborative efforts were made to develop effective means of collecting human sweat, particularly from physically active individuals under varying, controlled environmental conditions at USARIEM. Plans were made for a planning meeting of scientists from each of the cooperators' locations to be held in early FY08.

Scientific Publications: Log 115:

Approved: CHANDLER LAURENCE D Date: 09/06/2006

MICRONUTRIENT ABSORPTION AND METABOLISM

MANAGEMENT UNIT

5450-20-00

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Principal Investigator:** JANET ROSS HUNT

Start Date: 01/15/2004 **Term Date:** 01/14/2009

National Programs: 107 N Human Nutrition

Title: MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

Period Covered From: 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter?**

This is the annual report for the OSQR-approved project, 5450-51000-035-00D, "Mineral Utilization and Bioavailability in the 21st Century, with Changing Diets and Agricultural Practices". This research is directly related to three components of the ARS National Human Nutrition 107 research plan: (1) Human Nutrition Requirements, (6) Health Promoting Properties of Plant and Animal Foods, and (7) Bioavailability of Nutrients and Food Components.
Current trends and proposed changes in the US diet can substantially influence the mineral nutrient status of the population. Such changes include: a) an emphasis on plant-based diets with limited intake of red meat, b) mineral fortification or supplementation of diets proposed by health care professionals, or independently initiated by producers, and c) new technologies to produce genetically- or chemically- modified foods, or to stabilize and enhance the bioavailability of nutrients added to foods. The changes that may affect the mineral contents and/or the absorption and utilization (bioavailability) of dietary minerals, or disrupt the balance among interacting dietary minerals must be identified. Effective approaches such as dietary modifications, fortification/supplementation strategies, and agricultural and food production approaches must be identified to enhance human mineral nutrition and promote health.
The project has 3 objectives: (1) Determine how shifts in agricultural and dietary practices, such as the availability of functional/genetically modified foods and emphasis on plant-based diets with reductions in meat consumption will impact the intake, bioavailability, and dietary requirements of minerals. (2) Determine the effectiveness of current and proposed mineral fortification/supplementation practices for improving mineral nutrition while avoiding excessive or imbalanced mineral intakes. (3) Determine the mechanisms of uptake, transport, and retention of food minerals and how mineral nutritional status influences these mechanisms to impact the bioavailability of essential minerals, non-nutritive metals, and other food components.
This project will evaluate modifications that can enhance trace element nutrition, with emphasis on selenium (Se), iron (Fe), zinc (Zn), and copper (Cu). Agricultural practices that can influence the mineral contents of foods, especially Se, will be evaluated, with assessment of the health properties of such Se-enhanced foods. The influence of reduced dietary Fe and Zn bioavailability will be evaluated in human studies that will help define quantitative mineral needs, elucidate the effect of dietary phytate in combination with dietary Ca fortification, and evaluate the effectiveness of different forms of iron used for food fortification. An algorithm will be developed to assess the Zn bioavailability of diets from generally available food composition data. The basic mechanisms of uptake, transport, and retention of

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2006

food minerals will be evaluated to determine how mineral nutritional status and intake impact the absorption and metabolism of essential minerals, in interaction with non-nutritive metals and food components.
This research will provide critical information about the biological control of mineral absorption, of nutritionally important mineral-mineral interactions, and of bioavailability of minerals from various food and fortification sources. This knowledge is essential for the development of Dietary Reference Intakes, FDA food and dietary supplement regulations, and other U.S. nutrition and food policy guidelines for providing safe and adequate mineral nutrition from the food supply, and will be useful for the agricultural and food production sectors in the development of healthful foods.
The results of these studies will be useful to food producers for the development and promotion of healthy foods, and to health care officials and educators for the development of dietary advice that contributes to optimal nutrition. The ultimate beneficiary is the American consumer through policies and guidelines set by scientists and health care professionals based on these research findings and through transfer of enhanced agricultural products that improve nutrition worldwide.

2. **List by year the currently approved milestones (indicators of research progress)**

(This CRIS project began in January, 2004, and has now been in effect for 2.5 years)

Year 1 (Addressed in FY2005)
Complete 1st yr of wheat & buckwheat Se accumulation studies (1.1)
Complete transgenic wheat accumulation of Se study (1.1)
Develop cells lines and constructs for antioxidants & gene expression study (1.5)
Develop Se speciation methodology (1.1)
Complete interim blood analyses for Fe excretion study & write initial paper (1.9)
Enroll subjects in elemental Fe powder study (2.1)
Complete antibody prep & perform studies on up/down regulation of mineral transport in Caco-2 cells (3.1).
Complete studies on Cu transporter trafficking in Caco-2 cells given high Zn media (3.2).

Year 2 (Addressed in FY2006)
Complete 2nd yr of wheat & buckwheat Se accumulation studies (1.1)
Complete anti-oxidants & gene expression study (1.5)
Complete 1st yr of organic/conventional foods study (1.1)
Report transgenic wheat study (1.1)
Complete Se speciation in wheat study (1.1)
Develop Zn algorithm & prepare paper (1.7)
Enroll subjects in Zn requirement study (1.6)
Complete elemental Fe powders study (2.1)
Wrap up studies on up/down regulation of mineral transport in Caco-2 cells. Write manuscripts (3.1)
Wrap up studies on Cu transporter trafficking. Write manuscripts (3.2)

Year 3 (Addressed in FY2007)
Complete feeding portion of human high-Se beef study (1.3)
Report antioxidants & gene expression study (1.5)
Report wheat & buckwheat accumulation studies (1.1)
Complete 2nd yr of organic/conventional foods study (1.1)
Complete high-Se beef and aberrant crypt study (1.3)
Complete Zn requirement study (1.6)
Report elemental Fe powders study (2.1)
Complete studies on low mineral intakes, metal transporters, and cadmium

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2006

accumulation in intestinal cells (3.3)
Complete studies on relationship between Cu deprivation & Hephaestin activity (3.4)

Year 4 (Addressed in FY2008)
Complete analyses for human high-Se beef study (1.4)
Complete differences in bioavailability of mineral nutrients from organic/conventional broccoli (1.2)
Report high-Se beef and aberrant crypt study (1.3)
Complete study of phytate X Ca & Zn bioavailability (1.8)
Report Zn requirement study (1.6)
Complete study on validation of Caco-2 cell results with human absorption results on iron bioavailability from agricultural products (2.2)
Wrap up Cu/Heph/Fe absorption studies. Write manuscripts (3.4)
Complete study on marginal mineral & Cd exposure, analyze data (3.5).

Year 5 (Addressed in FY2009)
Report human high-Se beef study (1.4)
Complete comparison of high-Se foods aberrant crypt study (1.2)
Report comparison of high-Se foods and aberrant crypt study (1.3)
Report organic & conventional foods study (1.1)
Report study of phytate X Ca & Zn bioavailability (1.8)
Complete final report of Fe excretion data (1.9)
Report microencapsulated Fe study (2.2)
Wrap up studies in objective 3.5. Write manuscripts.
Complete new 5-year proposal.

3a. List the milestones (from the list in Question 2) that were scheduled to be addressed in FY 2006.

1. Complete 2nd yr of wheat & buckwheat Se accumulation studies (1.1)
   Milestone Fully Met

2. Complete anti-oxidants & gene expression study (1.5)
   Milestone Not Met
   Reason not met: Critical SY Vacancy
3. Complete 1st yr of organic/conventional foods study (1.1)
   Milestone Fully Met

4. Report transgenic wheat study (1.1)
   Milestone Not Met
   Reason not met: Critical SY Vacancy
5. Complete Se speciation in wheat study (1.1)
   Milestone Not Met
   Reason not met: Critical SY Vacancy
6. Develop Zn algorithm & prepare paper (1.7)
   Milestone Substantially Met

Project Number: 5450-51000-035-00D Accession: 0407991 FY: 2006

3a. 7. Enroll subjects in Zn requirement study (1.6)

Milestone Fully Met

8. Complete elemental Fe powders study (2.1)

Milestone Fully Met

9. Wrap up studies on up/down regulation of mineral transport in Caco-2 cells. Write manuscripts (3.1)

Milestone Substantially Met

10. Wrap up studies on Cu transporter trafficking. Write manuscripts (3.2)

Milestone Substantially Met

3b. **List the milestones (from the list in Question 2) that you expect to address over the next 3 years (FY2007, 2008 & 2009). What do you expect to accomplish, year by year, over the next three years under each milestone?**

Year 1 (Addressed in FY2007)
• Complete feeding portion of human high-Se beef study (1.3) High-selenium beef may be an excellent food for providing supplemental dietary selenium; a human feeding study will provide information concerning the bioavailability and potential health benefits of selenium from high-selenium beef. Samples have been collected and analyses will continue.
• Report antioxidants & gene expression study (1.5) Publications have been completed.
• Report wheat & buckwheat accumulation studies (1.1) Publications have been completed.
• Complete 2nd yr of organic/conventional foods study (1.1) This multi-year study will provide a robust data set that compares the nutritional profile of broccoli grown conventionally or by organic techniques and will help answer the question of whether organically grown vegetables have superior nutritional characteristics. Samples have been collected and publications completed.
• Complete high-Se beef and aberrant crypt study (1.3) Due to a critical SY vacancy, this milestone will not be completed.
• Complete Zn requirement study (1.6) This study will provide new information on the relationship between zinc intake, absorption, and absorptive adaptation to meet zinc requirements. Data will be analyzed.
• Report elemental Fe powders study (2.1) This new approach was not useful for assessing elemental iron powders. However, a resulting paper was published in 2006, providing unique data on the relationship of nonheme iron absorption with serum pro-hepcidin and ferritin in healthy women. This will be extended if possible with collaborations to specifically measure hepcidin in the collected samples.
• Complete studies on low mineral intakes, metal transporters, and cadmium accumulation in intestinal cells (3.3). The outcome of this study should provide basic information about mechanisms of marginal nutritional deficiencies such as zinc, iron, and calcium on the regulation of absorption of the toxic element cadmium, and provide useful information for setting policy on dietary allowances for cadmium exposure to humans.
• Complete studies on relationship between Cu deprivation & hephaestin activity (3.4). Completion of this study will provide valuable basic scientific information about dietary nutrient regulation of an important intestinal copper-dependent ferroxidase required for the efficient absorption of dietary iron.

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2006

Year 2 (Addressed in FY2008)
• Complete analyses for human high-Se beef study (1.4) This study will provide information concerning the bioavailability and potential health benefits of selenium from high-selenium beef.
• Complete differences in bioavailability of mineral nutrients from organic/conventional broccoli (1.2) This study will help answer the question of whether organically grown vegetables have superior nutritional characteristics. Publication has been completed.
• Report high-Se beef and aberrant crypt study (1.3) Due to a critical SY vacancy, this milestone will not be completed.

• Complete study of phytate X Ca & Zn bioavailability (1.8) This study will test the impact of a phytate X Ca X Zn interaction in practical human diets, and may be related to current recommendations to increase intake of whole grains and dairy products while reducing meat products. Samples have been collected and analyses will be completed.
• Report Zn requirement study (1.6) Publication will provide new information on the relationship between zinc intake, absorption, and absorptive adaptation to meet zinc requirements.
• Complete microencapsulated Fe study (2.2) Because the methodology for this study was tested and found to be ineffective with elemental iron powders, this planned milestone was replaced with a milestone to "Complete validation of Caco-2 cell results with human absorption results on iron bioavailability from agricultural products." That study was completed and a report has been submitted for journal publicaiton.
• Wrap up Cu/Heph/Fe absorption studies. Write manuscripts (3.4) Completion of this study will provide valuable basic scientific information about dietary nutrient regulation of an important intestinal copper-dependent ferroxidase required for the efficient absorption of dietary iron.
• Complete study on marginal mineral & Cd exposure, analyze data (3.5). Completion of this study will give insight into the mechanisms whereby mineral nutrient status of the mother can influence the transfer of the toxic element cadmium to the fetus.

Year 3 (Addressed in FY2009)
• Report human high-Se beef study (1.4) See above.
• Complete comparison of high-Se foods aberrant crypt study (1.2) Due to a critical SY vacancy, this milestone will not be completed.
• Report comparison of high-Se foods and aberrant crypt study (1.3) Due to a critical SY vacancy, this milestone will not be completed.
• Report organic & conventional foods study (1.1) The study has been reported.
• Report study of phytate X Ca & Zn bioavailability (1.8) This will report a test of the impact of a phytate X Ca X Zn interaction in practical human diets, and may be related to current recommendations to increase intake of whole grains and dairy products while reducing meat products.
• Complete final report of Fe excretion data (1.9) A single report will provide iron excretion data specific to women, enabling calculation of recommendations with less extrapolation from results of iron excretion in men.
• Report study on validation of Caco-2 cell results with human absorption results on iron bioavailability from agricultural products (2.2). This study will be useful in the further development and application of Caco-2 cell results for decisions on crop selection for biofortification. The paper has been prepared for journal submission.
• Wrap up studies in objective 3.5. Write manuscripts. Completion of this study will give insight into the mechanisms whereby mineral nutrient status of the mother can influence the transfer of the toxic element cadmium to the fetus.
• Complete new 5-year proposal.

Project Number: 5450-51000-035-00D Accession: 0407991 FY: 2006

4a. List the single most significant research accomplishment during FY 2006.

a. Dry bean consumption improves blood cholesterol: Consumption of ½ cup of cooked dried beans per day for three months caused no significant changes in fecal short chain fatty acid production or bacterial populations that were known to be specifically related to colon health. However, it was confirmed that the consumption of beans lowered serum total cholesterol and LDL in both normal volunteers and those with mild metabolic syndrome X, and that this would favor a benefit to cardiovascular health. These results provide data useful for setting dietary guidelines. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

4b. List other significant research accomplishment(s), if any.

b. Iron bioavailability from maize or beans; in vitro vs. in vivo results: A simple, economical method of assessing the bioavailability of iron from foods, estimating how well food iron is absorbed and utilized, is needed to evaluate and improve crop varieties to prevent human iron deficiency. An investigation with collaborators from ARS in Ithaca, NY, and the International Institute for Tropical Agriculture in Nigeria, determined that Caco-2 cell culture results correctly predicted ascorbic acid enhancement of iron bioavailability to humans from maize and great northern beans, but incorrectly predicted color-associated differences between bean varieties and their interaction with ascorbic acid. Further refinement of the cell culture model to correspond with the human results would provide an efficient and economical means of screening crop varieties for nutritional value to help prevent iron deficiency in developing regions of the world. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

c. Serum pro-hepcidin is not correlated with iron absorption in premenopausal women: Hepcidin, a recently discovered peptide with antimicrobial properties, is proposed to play a central role in the biological regulation of iron absorption. Iron absorption measured in 28 premenopausal women correlated inversely with serum ferritin, an indicator of body iron stores, but was unrelated to serum pro-hepcidin concentrations. This newly published research suggested limitations of a commonly used immunological assay of a hepcidin precursor, and emphasized the need to develop a more specific assay of serum hepcidin. A better understanding of the biological control of iron absorption will help in developing recommendations for iron intake, especially for people with chronic infection or inflammatory diseases. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

d. Ascorbic acid enhancement of iron absorption is not identical for all forms of iron: Ascorbic acid consumed in the same meal substantially enhances absorption of iron from food, but if an elemental iron powders used for food fortification is poorly absorbed, its absorption may also be less influenced by ascorbic acid. In research with 56 volunteers, ascorbic acid improved iron absorption from a breakfast cereal by four times if fortified with ferrous sulfate, but only doubled absorption when fortified with an electrolytic iron irradiated for the research. Although irradiation likely reduced absorption of the electrolytic iron, other iron powders that are poorly absorbed may also be less effectively enhanced by ascorbic acid, and this could be an important consideration when choosing iron fortificants to combat iron deficiency anemia. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

e. Providing excess iron to rats by diet or injections does not cure the anemia of

copper deficiency. Copper deficiency produces iron deficiency and anemia in rats and pigs by inhibiting an iron transport facilitator, hephaestin, in the intestinal cells. Doses of iron by diet or injections at three times the requirement had no effect on the signs of anemia induced in copper deficient rats. This research suggested that anemia of copper deficiency occurs primarily because of low iron absorption and inefficient loading of iron into transferrin because of very low copper-dependent ceruloplasmin activity. This then leads to inefficient delivery of iron to the erythroid cells for heme and hemoglobin synthesis. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

f. Selenium bioavailability varies in different mill fractions of wheat: Portions of cooked milled wheat fractions, flour, shorts, and bran containing selenium, were fed to rats. Bioavailability of selenium from flour was near 100%, from shorts, 85% and from bran, it was only 60%. These results indicate that high selenium wheat products, mainly those made from refined flour alone might be particularly well suited for use as a dietary selenium supplement. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

g. Greater mineral concentrations in historical varieties of wheat: Mineral analysis of wheat varieties grown under similar conditions showed that historical varieties were significantly higher in copper, iron, magnesium, manganese, phosphorus, selenium, and zinc than modern varieties. Results showed that modern wheat varieties require increased consumption of whole wheat bread to achieve the same percentage of the recommended dietary allowance (RDA) levels attainable by historical varieties high in mineral content. Selenium and iron concentrations were not associated with yield, suggesting the possibility for uncomplicated improvement, while weak to moderate negative correlations between yield and the other six minerals suggest a possible biological trade-off, and genetic improvement for these minerals may be more difficult to achieve. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components.)

5. **Describe the major accomplishments to date and their predicted or actual impact.**

The following accomplishments align with Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components:

- Demonstrated that the antioxidant activity of thioredoxin reductase is regulated by multiple dietary components, especially selenium and sulforaphane, and that silencing thioredoxin reductase results in greatly increased oxidative stress. An improved understanding of how diet influences oxidative stress will help determine dietary recommendations that can reduce the risk of chronic diseases such as cancer.

- Found that selenium accumulated by grains grown on high-selenium soils was highly bioavailable to humans and animals from processed wheat, but that selenium from buckwheat was less bioavailable to rodents than pure chemical sources of selenium. The selenium enhancement of grains is potentially useful for increasing dietary selenium intakes, with possible health benefits related to the risk of chronic diseases.

- Incorporated a selenium accumulation gene into wheat, and tested the effects of various growing conditions to increase the uptake of selenium by the transfected cultivars. (in collaboration with investigators at USDA/ARS, Albany, CA, and USDA/ARS, Houston, TX). Development of selenium accumulating strain of wheat may someday allow production of wheat with extremely high concentrations of selenium which could be used as a source of supplemental Se or as a selenium-fortificant in

cereal grain-based products.

- Developed methods to determine the chemical form of the selenium in foods, especially selenium in methylated forms that may be especially anticarcinogenic. The selenium compounds in dried vegetable powder were extracted and analyzed by HPLC coupled to inductively coupled plasma mass spectrometry. Further development of this methodology will allow us to better predict the bio-activity of selenium from numerous common plant foods.

- Demonstrated the potential antioxidant properties of selenium-enriched broccoli, which in rat diets was associated with decreased risk of some cancers. Extracts of selenium-enriched broccoli or of broccoli rich in sulforaphane effectively reduced DNA strand breaks in cultured liver cells of rats. Because DNA strand breaks are a major initiating event of many cancers, these results suggest that some of the cancer protective effects of sulforaphane and selenium may be protection against oxidative stress.

- Discovered a novel role for selenium in the up-regulation of cell cycle related genes that may lead to a better understanding of the essentiality of selenium as a nutrient and its involvement in cancer prevention.

- Demonstrated that, compared to a conventional farming technique, an organic farming method had limited influence on several nutritional characteristics of broccoli, including trace minerals, multiple individual glucosinolates, primary glucosinolate breakdown products, vitamin C and phenolic acids.

- Discovered that enhancing the selenium content of broccoli decreased total glucosinolate content, specifically sulforaphane, and changed the phenolic profile, especially reducing the content of hydroxy-cinnamic acids, suggesting that it may not be possible to simultaneously maximize all bioactive ingredients in a food, as enrichment with one compound may cause a concomitant decrease in another.

- Determined that zinc interference with copper transport appears unrelated to the expression of the human copper transporter, ATP7b. An understanding of the interaction between copper and zinc absorption will be useful in setting recommendations for balanced dietary intakes of the two elements.

- Confirmed that copper deficiency reduces iron absorption in rats of both sexes, resolving some mixed results from other laboratories. An understanding of the role of copper in iron absorption will be useful in setting recommendations for balanced dietary intakes and addressing the causes of nutritional anemias.

- Demonstrated that impaired iron absorption in copper deficient animals is associated with a reduction in the copper-containing hephaestin protein. Further understanding of the role of copper in iron absorption will be useful in resolving the problems of nutritional anemias that are not responsive to supplemental iron.

- Showed that in addition to impairing iron absorption, copper deficiency impairs red blood cell formation. An enhanced understanding of the role of copper in blood cell formation and hemoglobin synthesis will help set recommendations for dietary copper intakes.

- Discovered that marginal intakes of zinc, iron, and calcium greatly enhanced the accumulation of cadmium in the upper small intestine, independent of intestinal metallothionein concentrations, leading to a higher accumulation of the toxic metal

in the liver and kidneys. This suggests that populations with these nutrient deficiencies are especially susceptible to cadmium toxicity.

- Demonstrated that the 10% of people of Northern European origin who inherited the hemochromatosis mutation from one parent (heterozygous carriers) absorbed both heme and nonheme iron similar to those without the mutation, even from a meal highly fortified with iron and vitamin C, suggesting that current food iron fortification policies do not place this large group at increased health risk.

- Demonstrated that elemental iron powders commonly used to fortify staple foods with iron were less bioavailable to rats than iron from ferrous sulfate, and commercial versions differed considerably, suggesting that higher concentrations of these forms may be needed if they are used in international iron fortification programs.

- Showed that reduced and electrolytic iron sources were approximately 50 and 85% as effective as ferrous sulfate and 5 mg iron in the heme form was half as effective as 50 mg of iron from ferrous sulfate for improving body iron in premenopausal women. This research with humans will help to determine the most useful forms of iron to use in supplementation and fortification programs to reduced iron deficiency anemia worldwide.

The following accomplishment aligns with Human Nutrition Program 107, Component 4: Nutrient Requirements:

- In a sensitive assessment of iron excretion in women as well as men, found that iron excretion is unrelated to body iron status in men, but that it determines the iron status of pre-menopausal women, because of the substantial influence of menstrual iron losses. Such iron excretion data from women will contribute to setting dietary recommendations for women's iron intake, with less need to extrapolate from measurements in men.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

JR Hunt served as a member of the National Academies, Institute of Medicine, Food and Nutrition Board Committee on Mineral Requirements for Cognitive and Physical Performance of Military Personnel, April 2005-March, 2006, contributing to the report "Mineral Requirements for Military Personnel", Washington DC, National Academies Press, 2006.

JR Hunt participated in the National Academies, Institute of Medicine, Food and Nutrition Board workshop on DRI Research Recommendations, Washington DC, June 7-8, 2006, giving an invited presentation, "Fresh Perspectives: Research recommendations on DRIs for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc".

JR Hunt provided written consultation on a project concerning iron supplementation for the Pan-American Health Organization.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2006

In addition to presentations with the abstracts below, seminars were presented to potential collaborators at the Red River Valley Agricultural Research Center, Fargo, ND and to statistics students at a local high school. Three nutrition articles were written for the Grand Forks Herald.

Scientific Publications: **Log 115:**

1. Roughead, Z.K., Zito, C.A., Hunt, J.R. 2005. Inhibitory effects of dietary calcium on the initial uptake and subsequent retention of heme and nonheme iron in humans: comparisons using an intestinal lavage method. American Journal of Clinical Nutrition. 82:589-97. 0000171993
2. Fairweather-Tait, S., Lynch, Sean, Hunt, J.R., et al. 2005. The usefulness of in vitro models to predict the bioavailability of iron and zinc: a consensus statement from the HarvestPlus expert consultation. International Journal for Vitamin and Nutrition Research.75(6):371-374. 0000195689
3. Hunt, J.R. 2005. Dietary and physiological factors that affect absorption and bioavailability of iron. International Journal for Vitamin and Nutrition Research. 75(6):375-384. 0000179469
4. Hadley, K.B., Johnson, L.K., Hunt, J.R. 2006. Iron absorption by healthy women was not associated with either serum or urinary pro-hepcidin. American Journal of Clinical Nutrition. 84:150-5. 0000186142
5. Swain, J.H., Johnson, L.K., Hunt, J.R. 2006. An irradiated electrolytic iron fortificant is poorly absorbed by human subjects and is less responsive than FeSO4 to the enhancing effect of ascorbic acid. Journal of Nutrition. 136:2167-2174. 0000188682
6. Hunt JR. Bioavailability of iron, zinc and copper as influenced by host and dietary factors. In: Food and Nutrition Board, Institute of Medicine, National Academy of Sciences report on "Food and Nutrition Board: Institute of Medicine. Mineral Requirements for Military Personnel; Levels Needed for Cognitive and Physical Performance During Garrison Training. Washington, D.C.: National Academy Press, 2006, pp. 265-277. 0000182766
7. Finley, J.W. 2006. Bioavailability of selenium from foods. Nutrition Reviews. 64(3):146-151. 0000181014
8. Finley, J.W. 2005. Bioactive compounds and designer plant foods: the need for clear guidelines to evaluate potential benefits to human health. Chronica Horticulturae. 45(3):6-11. 0000181454
9. Reeves, P.G., Leary, P.D., Gregoire, B.R., Finley, J.W., Lindlauf, J.E., Johnson, L.K. 2005. Selenium bioavailability from buckwheat bran in rats fed a modified AIN-93g Torula yeast-based diet. Journal of Nutrition. 135:2627-2633. 0000182600
10. Reeves, P.G., Johnson, W.T. 2006. Copper. In: Driskell, J.A., Wolinsky, I., editors. Sports Nutrition: Vitamins and Trace Elements, 2nd Edition. Boca Raton, FL:CRC Taylor & Francis Group. p. 235-252. 0000170500
11. Keck, A., Finley, J.W. 2006. Aqueous extracts of selenium-fertilized broccoli increase selenoprotein activity and inhibit DNA single-strand breaks, but decrease the activity of quinone reductase in Hepa 1c1c7 cells. Food and Chemical Toxicology. 44:695-703. 0000174348
12. Keck, A.S., Finley, J.W. 2006. Database values for selenium do not reflect selenium contents of grain, cereals and other foods grown or purchased in the upper midwest of the United States. Nutrition Research. 26:17-22. 0000166136
13. Reeves, P.G., Demars, L.C. 2006. Signs of iron deficiency in copper-deficient rats are not affected by iron supplements administered by diet or by 0000193529

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2006

injection. Journal of Nutritional Biochemistry. 17:635-642.

14. Swain, J.H., Penland, J.G., Johnson, L.K., Hunt, J.R. 2006. Energy, mood and attention did not consistently improve with iron status in non-anemic women with moderate to low iron stores [abstract]. FASEB J. 20(4):A191. 0000187202

15. Hunt, J.R., Johnson, L.K. 2006. Iron excretion of healthy men and women, measured by isotope dilution [abstract]. FASEB J. 20(4):A194. 0000187181

16. Beisiegel, J.M., Glahn, R.P., Welch, R.M., Menkir, A., Maziya-Dixon, B.B., Hunt, J.R. 2006. A caco-2 cell model predicts relative iron absorption from tropical maize by women [abstract]. FASEB J. 20(4):A624. 0000187232

17. Beisiegel, J.M., Klevay, L.M., Hunt, J.R. 2006. Healthy postmenopausal women adapt to reduce zinc absorption in response to zinc supplementation [abstract]. FASEB J. 20(5):A985. 0000187209

18. Hadley, K.B., Hunt, J.R. 2006. Zinc regulation of skeletal matrix remodeling activities in growing rats [abstract]. FASEB J. 20(4):A626. 0000187197

19. Hunt, J.R. 2006. Absorption of nonheme, but not heme iron, is substantially reduced with high iron stores [abstract]. Journal of the American Dietetic Association. 106(8)S2:A-42. 0000193539

20. Finley, J.W. 2005. Selenium and sulforaphane from broccoli interact to alter gene expression and protection against oxidative stress in cultured cells [abstract]. Presented at the 230th ACS National Meeting, Washington DC, Aug 28 - Sep 1, 2005. 0000180378

21. Reeves, P.G., DeMars, L.C. 2006. Anemia in Cu-deficient rats was not reversed by administering high amounts of Cu-free Fe, either parenterally or by diet [abstract]. Journal of Federation of American Societies for Experimental Biology. 20(4):A193. 0000187378

22. Reeves, P.G., Saari, J.T. 2005. Bioavailability of copper from cooked dry beans [abstract]. Annals of Nutrition and Metabolism. 49(Suppl 1). p.2.3. 0000180163

23. Reeves, P.G., Finley, J.W. 2006. One-half cup of dry cooked pinto beans per day in addition to the regular diet lowered serum lipids in humans [abstract]. Presented by John W. Finley at Experimental Biology 2006, San Francisco, CA, March 31 - April 5, 2006. 0000192576

Approved: BLACKBURN WILBERT H **Date:** 09/26/2006

Project Number: 5450-51000-035-06S **Accession:** 0404307 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Principal Investigator:** JOHN W FINLEY

Start Date: 03/01/2001 **Term Date:** 09/15/2005

National Programs: 107 N Human Nutrition

Title: AGRICULTURAL PRODUCTION ASPECTS OF HIGH SELENIUM MEAT AND WHEAT

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-1-0310

Organization Name: NORTH DAKOTA STATE UNIVERSITY

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a specific cooperative agreement between ARS and North Dakota State University, Department of Animal Science. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

This project conducted comprehensive study of how high-Se wheat and meat are produced and utilized by animals. The Animal Science portion of the project was conducted at North Dakota State University.

Selenium-enriched beef may be an ideal means of supplementing dietary selenium. A series of studies demonstrated that it is possible to enrich beef with selenium by incorporating grains and hay grown on selenium-rich soils into feedlot rations of cattle. Studies demonstrated that selenium in beef can be increased from an average of 0.2 parts per million (ppm) to as much as 2.5 ppm. Diets containing up to 10 parts/million selenium did not alter carcass characteristics or feedlot performance measures such as feed efficiency and rate of gain. Cattle pastured on high selenium forages tended to select forage of better quality and higher selenium content than the average obtained by clipping all plant material. During pregnancy, selenium fed as selenomethionine accumulated in the fetus to a much greater extent that as selenocysteine, but the chemical form of selenium had no impact on any measure of pregnancy, gestation, or fetal health. Selenium enhancement of beef did not affect taste or other organo-sensory aspects, but selenium enhancement did slightly extend shelf life of retail cuts. These results demonstrated that increasing the selenium concentration does not adversely affect the consumer appeal of retail cuts of beef.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/06/2006

Project Number: 5450-51000-035-08S Accession: 0404351 FY: 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH Principal Investigator: JOHN W FINLEY

Start Date: 01/01/2001 Term Date: 06/30/2005

National Programs: 107 N Human Nutrition

Title: HEALTH BENEFITS OF HIGH-SELENIUM FOODS TO HUMANS

Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? Yes
Terminate in Two Months? No

Agreement Number: 58-5450-1-0315

Organization Name: OREGON STATE UNIVERSITY

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a specific cooperative agreement between ARS and Oregon State University, the Department of Environmental and Molecular Toxicology. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The collaborator at OSU has a long-standing interest in Se metabolism, and especially how different forms of Se affect the use and distribution of Se in the body, was a co-author of the IFAFS grant award that is being used to pay this Specific Cooperative Agreement and was instrumental in putting together a research agreement with the Chinese Academy of Preventive Medicine.

The distribution of selenium between proteins found in blood is an indication of how Se is metabolically partitioned in the body. Selenium is primarily found in three proteins: serum albumin, and the selenoproteins plasma glutathione peroxidase and plasma selenoprotein P. Ongoing collaborative studies with Oregon State University have been using a chromatographic method developed at OSU to separate these proteins. Samples from human trials, as well as from animal trials, have been analyzed by this procedure. The results are being incorporated into pertinent manuscripts, and will be reported in the parent CRIS 5450-51000-035-00D.

Scientific Publications: Log 115:

Approved: MCGUIRE MICHAEL R Date: 09/29/2006

Project Number: 5450-51000-035-09S **Accession:** 0404746 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Principal Investigator:** JOHN W FINLEY

Start Date: 06/01/2001 **Term Date:** 05/31/2006

National Programs: 107 N Human Nutrition

Title: HEALTH BENEFITS OF INTERACTING PHYTOCHEMICALS IN BROCCOLI

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-1-0330

Organization Name: UNIV OF ILLINOIS

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under specific cooperative agreement between ARS and the University of Illinois, Department of Nutritional Sciences. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The Principal Investigator at the University of Illinois has an active research program examining the health benefits of glucosinolate compounds found in broccoli. She has collaborated with a plant breeder to develop different strains of broccoli with different concentrations of glucosinolates. Our work with broccoli has examined the health benefits of selenium that accumulates in broccoli grown under special high-Se conditions. This project joined these two lines of research and to determine whether the two compounds have any nutritional interaction.

Selenium-enriched broccoli may be an excellent supplemental source of selenium because it contains chemical forms of selenium that protect against cancer but do not accumulate in the body as well as other forms of broccoli. A clinical study fed volunteers either 9 or 90 grams of broccoli either enriched or unenriched in selenium each day for 2 months. When compared to a similar study that fed subjects high-selenium wheat cereal, selenium from broccoli accumulated approximately 50% less than selenium from wheat, and more selenium from broccoli was excreted in the urine. These results support the hypothesis that selenium-enriched broccoli may supply cancer-inhibiting metabolites without inducing excessive accumulation in the body. In addition to results already published, any additional publications will be reported in the parent CRIS 5450-51000-035-00D.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/06/2006

Project Number: 5450-51000-035-13T Accession: 0405631 FY: 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH Principal Investigator: JANET ROSS HUNT

Start Date: 07/01/2002 Term Date: 09/30/2006

National Programs: 107 N Human Nutrition

Title: COMBATING IRON DEFICIENCY: ABSORPTION & EFFICACY IN HUMANS OF ELEMENTAL IRON POWDERS & HEME IRON

Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? Yes
Terminate in Two Months? No

Agreement Number: 03-5450-3-0237

Organization Name: COOPERATIVE STATE RESEARCH EDUCATION & EXTENSION SERVICE (CSREES), U.S. DEPARTMENT OF AGRICULTURE

Progress and Outcomes:

4d. Progress report.

This is the final report to document research conducted under a trust fund cooperative agreement between ARS and the USDA CSREES Nutritional Research Initiative competitive grant 2002-01885 (and tracked by CREES as CRIS 0192629) entitled "Combating Iron Deficiency: Absorption and Efficacy in Humans of Elemental Iron Powders and Heme Iron". This project had two objectives.

Objective 1 was to determine the efficacy of fortifying food with elemental iron powders, relative to equivalent amounts of ferrous sulfate, or of supplementing with a limited amount of iron in the heme form, on serum ferritin in women with low iron stores. In a randomized, blinded trial, 51 premenopausal women with moderate to low iron stores received daily for 12 wk: a) placebo; b) 5 mg iron as heme iron; or 50 mg iron as c) electrolytic iron; d) reduced iron; or e) FeSO4. Treatments were provided in 2 capsules (heme carrier) and 3 wheat rolls (other iron sources). Iron treatments did not affect food iron absorption. The 50 mg/d iron treatments increased fecal iron and free-radical generating capacity in vitro, but did not affect fecal water cytotoxicity. FeSO4 slightly increased fecal water content. Iron treatments did not affect most tests of mood, depression or attention, but the sensitivity of these comparisons was likely diminished by the broad range of iron status of the subjects, from marginal to moderate. Electrolytic iron was ~85% as efficacious as FeSO4 for improving body iron, but the power of this study was insufficient to detect any efficacy of the reduced or heme iron within 12 weeks. This demonstration of the usefulness of electrolytic iron powder as a food fortificant to improve iron status of women will be useful to developing nations as they develop food fortification policies that specify methods of iron fortification.

Objective 2 was to determine the absorption of irradiated electrolytic iron powder, relative to ferrous sulfate, as affected by dose and by interactions with ascorbic acid and phytic acid (3 experiments). Iron absorption by 56 volunteers was measured from a farina cereal breakfast radiolabeled with 59FeSO4 or an electrolytic 55Fe powder irradiated by neutron activation. Absorption of iron from the irradiated electrolytic powder was 5-15% that of FeSO4. Ascorbic acid (~160 mg) enhanced iron absorption from FeSO4 by 5 times vs. 2 times from electrolytic iron ($p < 0.01$ for interaction). Phytic acid from wheat bran inhibited iron absorption from FeSO4 and electrolytic iron by 73 and 50%, respectively (NS for interaction). Compared to 3

Project Number: 5450-51000-035-13T **Accession:** 0405631 **FY:** 2006

mg, a 20 mg dose reduced fractional absorption from FeSO4, but not electrolytic iron ($p< 0.0001$ for interaction). Despite a much higher bioavailability (50% relative to FeSO4) of this same electrolytic iron when previously tested in a pig model, the bioavailability of the irradiated electrolytic iron was poor when fed to humans. The diminished influence of ascorbic acid on the absorption of less soluble iron sources such as elemental iron powders may be an important consideration when choosing iron fortificants such as elemental iron powders.

Additional work tested the relationship between serum prohepcidin and iron absorption in healthy premenopausal women. Hepcidin is proposed as a regulator of iron absorption, but it has only been possible to immunologically measure the larger pro-peptide in serum. Serum pro-hepcidin concentrations were relatively stable within subjects, and correlated with serum ferritin. However, unlike serum ferritin, neither serum nor urinary pro-hepcidin concentrations were related to iron absorption in healthy women. Elucidation of the role of the antibacterial peptide hepcidin in regulating human iron absorption will help in understanding the interaction between iron absorption and infection, and may influence guidelines for iron fortification of populations with a high incidence of infection.

Further work evaluated the relationship of dietary iron and other dietary factors on human zinc absorption. A resulting bioavailability algorithm for zinc absorption from human diets explained ~83% of the variation in zinc absorption based on dietary content of zinc, phytic acid, calcium and iron. Readily available estimates of iron or zinc absorption from diets are useful to evaluate and improve the diets of populations at risk of zinc deficiency, and to evaluate the potential impact of changes in US diets.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/21/2006

Project Number: 5450-51000-035-16S **Accession:** 0405998 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Principal Investigator:** JANET ROSS HUNT

Start Date: 09/16/2002 **Term Date:** 09/30/2006

National Programs: 107 N Human Nutrition

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS TO SUPPORT RESEARCH ON HUMAN MINERAL NUTRITION

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-2-0335

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under Specific Cooperative Agreement between ARS and University of North Dakota. Additional details of the research can be found in the report for the parent CRIS project 5450-51000-035-00D.

To make dietary recommendations and evaluate dietary practices that promote good mineral nutrition for the population, there must be sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. The use of isotopic tracer methodology can effectively contribute to meeting these needs. Specifically, use of a whole body scintillation counter can safely and sensitively determine whole body retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach has the advantage of determining mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. It allows the use of a true "tracer" that does not alter the absolute mass of the mineral under investigation, and is easily and sensitively measured with minimal labor. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, applying an interdisciplinary approach to answering nutrition questions with whole body counting methodology. Accomplishments this year included health physicist support of investigations to assess bioavailability of iron and zinc using gamma-emitting radiotracers in human absorption studies. The health physicist led the work of the FDA-sanctioned UND Radioactive Drug Research Committee, which reviewed and provided oversight for all nutrition experiments employing radioactive tracers.

This year data analysis continued on the use of radioactive tracers to assess women's absorptive adaptation to different dietary zinc intakes from low to recommended levels, as a possible indicator of zinc requirements and to further assess women's absorptive adaptation to supplemental zinc. Human studies were conducted to assess the bioavailability of iron from different staple food varieties and to assess the interaction of calcium and phytic acid on zinc absorption.

Analyses of whole body counter data for regional bismuth-214 gamma ray emissions from humans collected over the past 18 years continued. It has been possible to combine these data with previously reported radon solubility in animal fat and fatty

Project Number: 5450-51000-035-16S **Accession:** 0405998 **FY:** 2006

tissues. This is of interest because bismuth-214 is a radioactive daughter of environmental radon-222. Radon-222 has been implicated in lung cancer dating back to 1576. However, it is not widely known that radon solubility in body fluids leads to a source of internal alpha radiation causing nano-injury at the cellular level. The intent of this preliminary work is to explore the role of environmental radon as a source of internal alpha injury and the onset of malignant or dysplastic changes of the organic structure as a healing response to the cellular injury. It is hypothesized that environmental radon may interact with dietary fat consumption leading to cellular damage in nerve tissue. A Rotterdam Study of 10,994 subjects concluded that a high intake of unsaturated fatty acids might protect against Parkinson disease. Furthermore, advanced age is associated with reduced brain levels of long-chain polyunsaturated fatty acids. A possible explanation for diseases associated with diets high in hydrogenated and saturated fats is storage of radon in these fatty acids. The decaying toxic, radioactive progeny, all confined to a localized area, deposit alpha and beta energy locally resulting in DNA and cellular damage. It is possible to speculate that Alzheimer's and Parkinson's Diseases result from an imbalance in the ratio of saturated fats to polyunsaturated fats in the brain. This imbalance might allow stored radon to accumulate in brain tissue, thereby producing bismuth-210 and polonium-210 species and localized nervous system damage. Study of the uptake and retention of such naturally occurring radioisotopes can improve our understanding of how diet and the environment may interact to affect health.

The sensitive detection of gamma-emitting isotopic tracers provides uniquely sensitive measurements of mineral retention in humans and animal models, as affected by nutritional status, dietary sources of nutrients, and genotype.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 10/16/2006

Project Number: 5450-51000-035-17T **Accession:** 0406927 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Principal Investigator:** PHILIP G REEVES

Start Date: 01/01/2003 **Term Date:** 09/30/2006

National Programs: 107 N Human Nutrition

Title: NUTRITIONAL VALUE OF BUCKWHEAT: TRACE ELEMENT VARIABILITY AND BIOAVAILIBILITY AND FAGOPYRITOL CONTENT

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-3-0406

Organization Name: MINN-DAK GROWERS, LTD.

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a trust fund cooperative agreement between ARS and MinDak Growers, Ltd., Grand Forks, ND. Additional details of the research can be found in the report for the parent project 5450-51000-035-00D. Publications will be cited under this parent CRIS.

The purpose of this agreement is to study the nutritional value of buckwheat, with emphasis on buckwheat as a source of nutritionally essential minerals. Minn-Dak Growers, Ltd., collected buckwheat and soil samples from buckwheat producers, and prepared those samples for analysis. The Grand Forks Human Nutrition Research Center (GFHNRC) conducted all chemical analyses, and conducted studies in rats to determine the bioavailability of minerals from buckwheat. North Dakota produces the majority of buckwheat grown in the US and much of that is grown in areas known to have high-selenium soils. A survey of buckwheat grown in these areas has shown it to contain between 0.5 and 1.0 micrograms selenium per gram, making it a nutritionally important source of selenium. However, the bioavailability of selenium from buckwheat is not known. To determine the bioavailability of selenium from buckwheat we repleted the selenium status of selenium-deficient rats with buckwheat enriched in selenium. Other rats were repleted with the pure the chemical forms of selenium, selenomethionine and selenite. After 49 days of selenium repletion, rats were killed and selenium status was determined by measuring selenoprotein activities and selenium concentrations in important tissues. Selenium from buckwheat was only 45-60% as bioavailable as selenomethionine and selenite. However, the average concentration of selenium in North Dakota buckwheat is almost twice the average concentration in US cereal grains, thus if consumed on an equal basis as other cereals, selenium-enriched buckwheat could be an important dietary source of selenium, despite slightly lower bioavailability.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 10/10/2006

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH **Principal Investigator:** JANET ROSS HUNT
Start Date: 09/29/2003 **Term Date:** 09/28/2008
National Programs: 107 N Human Nutrition
Title: HUMAN STUDIES RESEARCH
Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No
Agreement Number: 58-5450-3-0324
Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter?**

   To improve the nation's nutrition and health (Goal 4 of the Agricultural Research Service Strategic Plan, 2003-2007), research is necessary to generate new knowledge in human nutrition, to improve the understanding of optimal nutrient requirements for known and new classes of nutrients at all stages of the life cycle, and to better understand relationships between diet and health. Although much can be learned using basic biochemical, cellular, and animal models of human nutrition, some questions require direct human studies to assure that the results are applicable to humans.

This agreement between ARS and the University of North Dakota (UND) is based on a mutual interest in human nutrition as it pertains to the maintenance of optimum health, including reduction of disease risk. The objective of the agreement is to investigate the role of nutrients in human health, to determine their bioavailability from foods and mixed diets, to investigate their biological activities in cancer prevention, in bone and joint health, in cardiovascular health, and in physiological and psychological development and function. Diets designed to contain known amounts of essential and non-essential nutrients, or foods containing specific nutrients or other bioactive components, are provided to human volunteers under controlled conditions of dietary intake, so that their effects on clinical chemical (blood constituents), physiological (blood pressure, cardiovascular function respiratory function, neuro-muscular function), neurological (mood, neurologic function), urinary and fecal excretion, and other measures of biological activity and health status may be determined. Human studies include residential, non-residential and field-based investigations using such approaches as dietary recall, metabolic balance, radio/stable isotope retention, physiological/neurological function assessment, and specific metabolic/enzyme analyses. Principal Investigators for UND are from the Department of Physics and Department of Psychology. UND provides an Institutional Review Board to review all protocols for studies involving human subjects at the Grand Forks Human Nutrition Research Center, provides the competent personnel to participate in the planning and execution of studies, collaborates with ARS on related animal model studies, and provides peer review of scientific manuscripts.

This research is directly related to five components of the ARS National Human Nutrition 107 research plan: (1) Human Nutrition Requirements, (2) Diet, Genetics,

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2006

Lifestyle, and the Prevention of Obesity and Disease, (5) Health Promoting Intervention Strategies for Targeted Populations (6) Health Promoting Properties of Plant and Animal Foods, and (7) Bioavailability of Nutrients and Food Components. The research provides knowledge to policy makers and other scientists for setting guidelines to improve human nutrition and reduce disease risk.

2. List by year the currently approved milestones (indicators of research progress)

This specific cooperative agreement provides support for human studies. Specific milestones are documented in the CRIS projects cited above. In support of those milestones, this report addresses milestones for conducting key Grand Forks Human Nutrition Research Center (GFHNRC) human studies with approval by the UND IRB.

Year 1 (FY2004)

Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
006 Human absorption of iron fortification sources assessed using an isotope displacement method
007 Beef as a component of a healthy diet: does it improve selenium and trace element status of healthy men and women?
049 Body iron excretion
144 Effects of low, moderate and high zinc intakes on copper nutriture
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
Complete data collection for approved studies
064 Meat protein and calcium: Do they interact synergistically or antagonistically?
098 Effect of daily intake of wheat cereal containing various concentrations of selenium on selenium status in humans
501 Improved health in humans consuming broccoli high in sulforaphane and selenium
404 Physical health, fitness, nutrition, and mental health in Northern Plains Indians

Year 2 (FY2005)

Collaborate in planning and approvals for human studies
008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids
042 Effects of calcium and phytate on zinc absorption
095 Iron absorption from agricultural products
298 Micro foundations of health and economic development: The impact of iron supplementation on social and economic prosperity in Indonesia
Recruit subjects and begin or continue data collection for approved studies
006 Human absorption of iron fortification sources assessed using an isotope displacement method
008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids
049 Body iron excretion
095 Iron absorption from agricultural products
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
Complete data collection for approved studies
007 Beef as a component of a healthy diet: does it improve selenium and trace element status of healthy men and women?

Project Number: 5450-51000-035-18S Accession: 0407722 FY: 2006

090 Determining dietary zinc requirements from adaptation in zinc absorption
144 Effects of low, moderate and high zinc intakes on copper nutriture

Year 3 (FY2006)

Collaborate in planning and approvals for human studies
009 Predicting dietary selenium needs to achieve target blood selenium levels
303 Estimating mineral nutrient requirements by cross-sectional statistical analyses of existing data
Recruit subjects and begin or continue data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels
042 Effects of calcium and phytate on zinc absorption
095 Iron absorption from agricultural products
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
601 Nutrition effects on cognitive performance of young versus elderly community-living adults
Complete data collection for approved studies
008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids
042 Effects of calcium and phytate on zinc absorption
095 Iron absorption from agricultural products
298 Micro foundations of health and economic development: The impact of iron supplementation on social and economic prosperity in Indonesia

Year 4 (FY2007)
Collaborate in planning and approvals for human studies
312 Physical activity and weight of YMCA members.
603 Formative evaluation of a preschool children obesity prevention program.
604 Barriers and facilitators to healthy eating and activities on a college campus.
Recruit subjects and begin or continue data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels.
205 Predication and validation of soldier fluid requirements when wearing body armor.
303 Estimating mineral nutrient requirements by cross-sectional statistical analyses of existing data.
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
312 Physical activity and weight of YMCA members.
603 Formative evaluation of a preschool children obesity prevention program
604 Barriers and facilitators to healthy eating and activities on a college campus
605 Copper deficiency as a predictor of pre-eclampsia
Complete data collection for approved studies
601 Nutrition effects on cognitive performance of young versus elderly community-living adults

Year 5 (FY2008)
Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
303 Estimating mineral nutrient requirements by cross-sectional statistical analyses of existing data
605 Copper deficiency as a predictor of pre-eclampsia
Complete data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2006

403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
312 Physical activity and weight of YMCA members.
603 Formative evaluation of a preschool children obesity prevention program
604 Barriers and facilitators to healthy eating and activities on a college campus

3a. List the milestones (from the list in Question 2) that were scheduled to be addressed in FY 2006.

1. Collaborate in planning and approvals for human studies:
   009 Predicting dietary selenium needs to achieve target blood selenium levels

   Milestone Fully Met
2. 303 Estimating mineral nutrient requirements by cross-sectional statistical analyses of existing data

   Milestone Fully Met
3. Recruit subjects and begin or continue data collection for approved studies:
   009 Predicting dietary selenium needs to achieve target blood selenium levels

   Milestone Fully Met
4. 042 Effects of calcium and phytate on zinc absorption

   Milestone Fully Met
5. 095 Iron absorption from agricultural products

   Milestone Fully Met
6. 403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?

   Milestone Fully Met
7. 601 Nutrition effects on cognitive performance of young versus elderly community-living adults

   Milestone Fully Met
8. Complete data collection for approved studies:
   008 Beans and colon health in humans: Effect of resistant starch from beans and changing the lower bowel bacterial populations and the production of short chain fatty acids

   Milestone Fully Met
9. 042 Effects of calcium and phytate on zinc absorption

   Milestone Fully Met
10. 095 Iron absorption from agricultural products

    Milestone Fully Met
11. 298 Micro foundations of health and economic development: The impact of iron supplementation on social and economic prosperity in Indonesia

    Milestone Fully Met

Project Number: 5450-51000-035-18S Accession: 0407722 FY: 2006

3b. List the milestones (from the list in Question 2) that you expect to address over the next 3 years (FY2007, 2008 & 2009). What do you expect to accomplish, year by year, over the next three years under each milestone?

(FY2007)
Collaborate in planning and approvals for human studies (Specific objectives are added in support of the CRIS research projects or associated, externally-funded projects as planned and approved through the UND Institutional Review Board)
312 Physical activity and weight of YMCA members. By determining the incidence of overweight (BMI >25) in people who exercise habitually, this project will facilitate future research to identify behavioral patterns (diet and physical activity) that may promote a healthy weight.
603 Formative evaluation of a preschool children obesity prevention program. By developing and assessing the feasibility of a preschool obesity prevention program utilizing education and physical activity targeting the child and the child's caregiver, this project will help define useful approaches to combat childhood obesity.
604 Barriers and facilitators to healthy eating and activities on a college campus. An understanding of the barriers and facilitators to healthy eating and increased physical activity by university students will help in the development of obesity prevention programs.
Recruit subjects and begin or continue data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels. An assessment of the relationship between selenium supplement dose and blood selenium response will be useful for developing recommendations for selenium supplementation to reduced cancer risk.
205 Predication and validation of soldier fluid requirements when wearing body armor. This assessment in collaboration with the US Army will provide data to improve recommendations for fluid intakes of US soldiers.
303 Estimating mineral nutrient requirements by cross-sectional statistical analyses of existing data. Data combined from past mineral balance studies will have substantial statistical power to evaluate trace mineral requirements and indices of marginal status in healthy adults.
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women? This 2-y supplementation study will determine whether zinc and copper, in addition to calcium supplementation, will increase bone mineral density.
312 Physical activity and weight of YMCA members. (see above)
603 Formative evaluation of a preschool children obesity prevention program. (see above)
604 Barriers and facilitators to healthy eating and activities on a college campus. (see above)
605 Copper deficiency as a predictor of pre-eclampsia. This study will evaluate possible associations between marginal copper deficiency and pre-eclampsia of pregnancy.
Complete data collection for approved studies
042 Effects of calcium and phytate on zinc absorption. This study will determine the interaction of dietary calcium and phytate on zinc absorption in practical human diets.
601 Nutrition effects on cognitive performance of young versus elderly community-living adults. This study will evaluate age-related changes in memory performance, as modified by factors including dietary intake and blood nutrient concentrations.

(FY2008)
Collaborate in planning and approvals for human studies

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2006

Recruit subjects and begin or continue data collection for approved studies
303 Estimating mineral nutrient requirements by cross-sectional statistical analyses of existing data. (see above)
605 Copper deficiency as a predictor of pre-eclampsia. (see above)
Complete data collection for approved studies
009 Predicting dietary selenium needs to achieve target blood selenium levels (see above)
403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women? (see above)
312 Physical activity and weight of YMCA members. (see above)
603 Formative evaluation of a preschool children obesity prevention program. (see above)
604 Barriers and facilitators to healthy eating and activities on a college campus. (see above)

4a. List the single most significant research accomplishment during FY 2006.

a) New Estimation of the Magnesium Requirement for Men and Women: A substantial portion of the population fails to meet the current recommended dietary allowance for magnesium. Magnesium requirements for healthy men and women, assessed using magnesium balance data from 27 human controlled feeding studies, were considerably lower than previously estimated. These results can be useful in revising intake recommendations for magnesium. (Human Nutrition Program 107, Component 4: Nutrient requirements. See additional information in the report for CRIS 5450-51000-034-00D).

4b. List other significant research accomplishment(s), if any.

b) Dry bean consumption improves blood cholesterol: The consumption of ½ cup of cooked dried beans per day for three months lowered serum total cholesterol and LDL in both normal volunteers and those with mild metabolic syndrome X, a possible benefit to cardiovascular health. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components. See additional information in the report for CRIS 5450-51000-035-00D.)

c) Iron bioavailability from maize or beans; in vitro vs. in vivo results: Caco-2 cell culture results correctly predicted ascorbic acid enhancement of iron bioavailability to humans from maize and great northern beans, but incorrectly predicted color-associated differences between bean varieties and their interaction with ascorbic acid. Further refinement of the cell culture model to correspond with the human results would provide an efficient and economical means of screening crop varieties for nutritional value to help prevent iron deficiency in developing regions of the world. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components. See additional information in the report for CRIS 5450-51000-035-00D.)

d) Nutrient composition of traditional American Indian plant foods: Traditional American Indian plant foods were collected in a culturally respectful and sensitive manner, received and processed by the ARS Nutrient Data Laboratory (Beltsville Human Nutrition Research Center), and analyzed by contract laboratories. The results were shared with leaders of the participating tribes, and will contribute to a national nutrient database of foods from American Indians and Alaska Natives and to planning culturally-sensitive interventions to prevent obesity, diabetes and cardiovascular disease in this population. (Human Nutrition Program 107, Component 6 Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Component 7 Lifestyle and Health-Promoting Intervention Strategies for Targeted Populations. See additional information in the report for CRIS 5450-51530-009-00D.)

Project Number: 5450-51000-035-18S Accession: 0407722 FY: 2006

4c. List significant activities that support special target populations.

a) The research center continued to work with American Indians to develop successful partnerships and to promote research on health promotion. Examples include participation in the 5th Annual American Indian Research Forum as part of the University of North Dakota's Indian Association Timeout Celebration, initiation of a Cultural Awareness Workshop at United Tribes Community College, and establishment of agreements with Cankdeska Cikana Community College (Spirit Lake Reservation) and the University of North Dakota to work together to promote health and prevent obesity and diabetes among American Indians in the Northern Great Plains. (Human Nutrition Program 107, Component 6 Prevention of Obesity and Disease: Relationship between Diet, Genetics, and Lifestyle. See report for CRIS 5450-51530-009-00D.)

4d. Progress report.

This report serves to document research conducted under a specific cooperative agreement between ARS and the University of North Dakota. Additional details of research can be found in the report for the parent project 5450-51000-035-00D.

5. Describe the major accomplishments to date and their predicted or actual impact.

Determined that serum pro-hepcidin, a precursor of the hepcidin molecule hypothesized to regulate iron absorption, was not associated with iron absorption in premenopausal women. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components. See report for CRIS 5450-51000-035-00D.)
Determined that the reduced bioavailability (associated with reduced solubility) of some elemental iron powders can also reduce the enhancing effects of ascorbic acid on their absorption. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components. See report for CRIS 5450-51000-035-00D).

Found that 20 mg zinc supplementation, 5 days/week for 10 weeks, improved visual memory reaction times, word recognition, and vigilance in cognitive testing of 7th grade students. (Human Nutrition Program 107, Component 4: Nutrient Requirements. See report for CRIS 5450-51530-009-00D).

Discovered that a high, compared with a low meat diet improved calcium retention in postmenopausal women when calcium intake was low, but had minimal influence when calcium intake was high. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components. See report for CRIS 5450-51000-034-00D).

Determined that soy protein (substituted for meat protein) had a negligible effect on calcium retention by postmenopausal women. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components. See report for CRIS 5450-51000-034-00D).

Determined that iron absorption of fortified foods did not differ significantly between genetic carriers (~10% of US population) of a mutation associated with hemochromatosis and those without the mutation. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components. See report for CRIS 5450-51000-035-00D).

Showed that ferrous sulfate, reduced, electrolytic, and heme iron can all improve body iron status in premenopausal women. (Human Nutrition Program 107, Component 2: Bioavailability of Nutrients and Food Components. See report for CRIS 5450-51000-035-00D).

Conducted a survey assessing Native American health, nutrition and physical activity

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2006

and distributed summaries of the findings to participating Indian tribes and communities. (Human Nutrition Program 107, Component 7: Health Promoting Intervention Strategies for Targeted Populations. See report for CRIS 5450-51530-009-00D).

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

The accomplishments are documented in manuscripts in peer reviewed scientific journals, and in presentations at scientific and public meetings. See the reports for the CRIS projects listed above.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

See the reports for the CRIS projects listed above.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/25/2006

Project Number: 5450-51000-035-19S **Accession:** 0408261 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH **Principal Investigator:** GERALD F COMBS
Start Date: 04/16/2004 **Term Date:** 03/15/2008
National Programs: 107 N Human Nutrition
Title: DEVELOPMENT OF RAPID SELENIUM ANALYSIS TECHNOLOGY
Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No
Agreement Number: 58-5450-4-0346
Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a specific cooperative agreement between ARS and the University of North Dakota. Additional details of research can be found in the report for the parent project 5450-51000-035-00D.

Work was conducted in the cooperators laboratory with local support from North Dakota agricultural interests to perfect the prototype for the rapid selenium analyzer. The most important feature of this prototype is the rapid liquification of a coarsely ground sample of grain using high-pressure steam. This process, developed in this project, can reduce within 10 minutes a grain sample pre-processed in a coffee mill to a clear liquid that can be subjected to automated chemistry and instrumental analysis for total selenium content. The entire analysis takes 50-55 min and has the potential to be miniaturized to a version the feasibility of which can be evaluated in the field, for example in a grain mill or elevator.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/25/2006

Project Number: 5450-51000-035-22T **Accession:** 0408646 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: MARY J KRETSCH **Principal Investigator:** PHILIP G REEVES
Start Date: 05/01/2004 **Term Date:** 04/30/2009

National Programs: 107 N Human Nutrition

Title: HIGH SELENIUM PINTO BEANS AS A VALUE-ADDED PRODUCT

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 04-5450-4-0418

Organization Name: NORTHARVEST BEAN GROWERS ASSOCIATION

Progress and Outcomes:

4d. **Progress report.**

This report serves to document research conducted under a Trust Fund Cooperative Agreement between ARS and NorthHarvest Bean Growers, Ltd. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D. The purpose of this agreement was to determine factors that affect the accumulation of selenium in pinto beans. Dry edible beans are a major crop raised in North Dakota, and there may be substantial health benefits associated with consumption of beans. Moreover, beans raised in North Dakota may contain much higher concentrations of selenium, a nutrient that may help prevent cancer. Representative samples of harvested pinto beans obtained from more than 70 locations in five distinct geographical regions of North Dakota were analyzed for selenium and other major mineral elements. The average content of selenium was approximately 5-fold the U.S. average, but there was great variation, even within a small geographical area. Modeling procedures were used to determine variables that may be predictive of selenium content; the project was extended over a second growing season.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/25/2006

Project Number: 5450-51000-035-23T **Accession:** 0408799 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Principal Investigator:** JANET ROSS HUNT

Start Date: 07/15/2004 **Term Date:** 03/31/2006

National Programs: 107 N Human Nutrition

Title: COMPARISON OF IRON BIOAVAILABILITY TO HUMANS FROM TWO STRAINS OF CORN USED IN NIGERIA

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 05-5450-5-0171

Organization Name: INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE

Progress and Outcomes:

4d. Progress report.

This report serves to document research conducted under a trust fund cooperative agreement between ARS and the International Institute for Tropical Agriculture in Nigeria ("Comparison of iron bioavailability to humans from two strains of corn used in Nigeria"). Additional details of research can be found in the report for the parent project 5450-51000-035-00D. This investigation was conducted with collaborators from ARS in Ithaca, NY, and the International Institute for Tropical Agriculture in Nigeria.

The objective of this project was to evaluate the bioavailability to humans of iron from two strains of Nigerian corn, and to demonstrate the enhancement of iron bioavailability by increasing the ascorbic acid content of a Nigerian corn-based meal. An additional objective was to confirm previous in vitro iron bioavailability differences between two maize varieties, directly comparing human and Caco-2 cell results. A randomized, 2 x 2 factorial, 29-d experiment compared women's iron absorption (n=26) from two maize varieties (ACR vs. TZB). Each food was served with and without orange juice to provide ascorbic acid. Nonheme iron bioavailability was determined from 2-wk whole body and erythrocyte retention of extrinsically added radioiron tracers, and compared with Caco-2 cell results from identical meals. In contrast to results with previous harvests, in vitro results predicted no difference in iron availability between the maize varieties. Maize varieties did not affect percent iron absorption, but women tended ($p = 0.06$) to absorb 4-7 µg more iron/meal from ACR compared to TZB, because of a slightly higher corn iron content (0.8 vs 0.7 mg/meal, respectively, $p < 0.0001$). As predicted in vitro, ascorbic acid increased iron absorption from maize by at least 3 times ($p<0.0001$). Caco-2 cell results correctly predicted ascorbic acid enhancement of iron bioavailability to humans from maize. These results were presented in abstract form at an experimental biology research meeting, and a manuscript was prepared for journal submission. When published, the citation will be reported in the report for the parent CRIS project 5450-51000-035-00D.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/06/2006

Project Number: 5450-51000-035-24R **Accession:** 0409137 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Principal Investigator:** PHILIP G REEVES

Start Date: 11/16/2004 **Term Date:** 05/31/2006

National Programs: 107 N Human Nutrition

Title: BEANS AND COLON HEALTH IN HUMANS

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 05-5450-5-0403

Organization Name: MICHIGAN STATE UNIVERSITY

Progress and Outcomes:

4d. **Progress report.**

This is the final report to document research conducted under a reimbursable agreement between ARS and Michigan State University, Beans for Health Alliance. Additional details of this research can be found in the report for the parent project 5450-51000-035-00D.

Consumption of beans may enhance fermentation in the lower bowel in a manner that helps to inhibit colon cancer, however there is very little experimental evidence in humans to support this hypothesis. A human study was initiated in which 40 male and female volunteers consumed 200 g of beans/day, and another 40 consumed chicken soup as controls. One-half of the volunteers were healthy adults and one-half had signs and symptoms of metabolic syndrome X, which could lead to cardiovascular disease. Blood was collected to test for changes in lipid profiles. Feces were collected from each subject and used to inoculate an in-vitro fermentation system that was later analyzed for volatile fatty acids. Analyses of the data showed that individuals who consumed beans had significantly lower total cholesterol, HDL, and LDL than those who consumed chicken soup. Fecal propionate production was significantly increased in volunteers who consumed beans. This might have a positive influence on the reduction of inflammation in the lower bowel. In addition, the consumption of beans lowered serum total cholesterol and LDL in both normal volunteers and those with mild metabolic syndrome X, and that this may benefit cardiovascular health A summary report was provided to the beans for Health Alliance, and additional manuscripts in Scientific Journals will be reported with the parent project 5450-51000-035-00D.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/21/2006

Project Number: 5450-51000-035-25T **Accession:** 0409464 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM

NPL Leader: MARY J KRETSCH **Principal Investigator:** JANET ROSS HUNT

Start Date: 04/01/2005 **Term Date:** 07/31/2006

National Programs: 107 N Human Nutrition

Title: MICRO FOUNDATIONS OF HEALTH AND ECONOMIC DEVELOPMENT: THE IMPACT OF IRON SUPPLEMENTATION ON SOCIAL AND ECONOMIC PROSPERITY IN INDONESIA

Period Covered **From:** 10/ 2005 **To:** 9/ 2006 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 05-5450-5-0415

Organization Name: RAND CORPORATION

Progress and Outcomes:

4d. **Progress report.**

This is the final report to document research conducted a trust fund cooperative agreement between ARS and the Rand Corporation ("Micro foundations of health and economic development: The impact of iron supplementation on social and economic prosperity in Indonesia"). Additional details of research can be found in the report for the parent project 5450-51000-035-00D.

The objective of this project is to provide biochemical assessment of iron and nutrient status, through analysis and assessment of approximately 1200 blood samples, to help assess the impact of iron supplementation on family economic status in Indonesia. Human studies approvals were obtained, and the blood samples were analyzed. The results are being analyzed, as they relate to additional health and economic data, by the collaborators at UCLA and the RAND corporation. Future publications will be reported in reports for the parent project 5450-51000-035-00D.

Scientific Publications: **Log 115:**

Approved: MCGUIRE MICHAEL R **Date:** 09/21/2006

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: DAVID M KLURFELD **Principal Investigator:** ERIC O UTHUS
Start Date: 07/21/2004 **Term Date:** 04/30/2009

National Programs: 107 N Human Nutrition

Title: ROLE OF DIETARY SELENIUM ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

Period Covered From: 10/ 2005 **To:** 9/ 2006 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter?**

Improving the diet by increasing the consumption of whole grains, fruits and vegetables may decrease the incidence of cancer by 30-40%. Although fiber, vitamins and phytochemicals have received the most attention as chemopreventive components of a diet rich in grains, fruits and vegetables, minerals also may be important chemopreventive components. For example, human epidemiologic and supplementation studies, as well as extensive animal studies, have shown the efficacy of selenium in cancer prevention. Food contains different chemical forms of selenium as well as other dietary constituents which will influence the chemopreventive effect of selenium. Furthermore, recent studies suggest that dietary copper protects against colon cancer in several animal models. Other dietary minerals may be beneficial but their role in cancer prevention has not been thoroughly investigated. Mammary, colon and prostate cancers are the main types of cancer which are influenced by dietary factors. A key to understanding the relationship between optimal mineral intake and cancer is determining the effects of mineral intake on cellular processes such as gene expression, oxidative stress, apoptosis and signal transduction. Studies are and will be conducted to determine whether mineral elements such as selenium, copper, and zinc affect biomarkers of carcinogenesis, including carcinogen-induced aberrant crypt formation (a preneoplastic lesion for colon cancer), carcinogen-DNA adduct formation, oxidative status, selenoprotein and detoxifying enzyme activities, DNA methylation and tumor development. Min (multiple intestinal neoplasia) mice will be used to study the effects of trace minerals on the pathogenesis of intestinal cancer in a genetic model for cancer susceptibility. These mice contain a mutation in the murine homolog of the human APC gene and develop spontaneous tumors throughout the intestine. Several observations implicate a role for altered DNA methylation in cancer pathogenesis: the global level of DNA methylation is generally lower but there is gene specific hypermethylation and DNA methyltransferase activity is usually higher in tumor cells than in normal cells. Global DNA methylation, gene specific DNA methylation, methyl metabolism and DNA methyltransferase activity will be evaluated in colon-derived human cells cultured in medium containing different chemical forms of selenium and different concentrations of folate, iron, or zinc and in animals fed diets containing different amounts of selenium, folate, iron or zinc. To determine the mechanisms for the chemopreventive effects of selenium and copper against colon cancer, gene specific macroarrays will be utilized and the effects of copper and selenium on signal transduction pathways for apoptosis and regulation of the cell cycle will be examined in cultured cells. The biological activity of zinc transcription factors will be studied using electrophoretic mobility shift assays or reporter gene constructs. Controlled human feeding studies and/or supplementation studies have and will be conducted to determine whether trace minerals shown to

affect carcinogenesis in animal and cell culture models affect cancer susceptibility in humans. Humans will be fed different diets and fecal water will be analyzed for cytotoxicity, apoptosis, genotoxicity, free radical production and alkaline phosphatase activity. Lymphocytes will be analyzed for DNA methylation, expression of cancer related proteins and measures of oxidative stress/status. Serum from animals or humans fed different concentrations of trace minerals will be used in cell culture systems to investigate cancer susceptibility.

Cancer is the second leading cause of death in the United States. It has been estimated that the cost for the treatment and care of this disease is approaching $200 billion per year. In addition to the economic impact, the development of cancer may prevent many from enjoying life to its fullest. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 30-40% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake are factors that can affect cancer susceptibility. Thus, providing information about requirements and factors that affect those requirements of mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

This research is related to National Program 107, Human Nutrition. The research addresses Performance Goal 3.1.1 of the National Program Action Plan: Human Nutrition Requirements. This research is relevant to Component 1: Nutrient Requirements because one of the priority objectives is to adapt current methods or develop new methods to identify specific disease preventing bioactive dietary factors and elucidate their mechanisms of action. Another priority objective is to use the biomarkers as screening tools to identify the specific bioactive factor(s) responsible for the effects. This research is also relevant to Component 2: Diet, Genetics, Lifestyle and the Prevention of Obesity and Disease. This research will identify the nutrient-relevant influences on gene expression that have consequences on human health and disease.

Several trace minerals have been demonstrated to reduce the risk of developing several types of cancer, but the mechanism by which this occurs is unknown. These studies will address this problem, and thus are of interest to health professionals and policy makers. Understanding the mechanism by which trace elements inhibit cancer has the potential to impact recommendations of how much of the dietary trace mineral should be consumed daily; this in turn has the potential to impact how the medical establishment approaches cancer prevention and how the food industry prepares and/or fortifies specific foods.

2. **List by year the currently approved milestones (indicators of research progress)**

Year 1 (FY 2005)

Initiate and optimize the HT 29 cell culture studies to determine whether cellular selenium status regulates the cyclin-dependent kinase pathway through the c-Myc gene (1.1).

Initiate and optimize experiments to determine whether methylselenol causes colon cell cycle arrest and induces differential gene expression in the cyclin-dependent kinase pathway (1.3).

Develop constructs and conduct experiments to determine whether thioredoxin reductase (TR) is regulated by selenium availability (dose and chemical form) as well as by ARE inducers and by oxidative stress (2.1 & 2.2).

Determine whether phytochemicals in broccoli act synergistically with selenium in increasing TR activity (2.2).

Project Number: 5450-51000-036-00D Accession: 0408616 FY: 2006

Complete the animal portion of the experiment to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression. Initiate metabolite and enzyme assays (2.6).

Complete animal portion of experiment to study the interaction between selenium and folate. Initiate metabolite and enzyme assays. Begin restriction length genome scanning (RLGS) on select samples (3.3).

Complete and report RLGS on initial (pilot) Ames dwarf mouse study (pre-3.2).

Year 2 (FY 2006)

Finish data collection on the Caco-2 cell culture studies designed to determine whether cellular selenium status regulates the cyclin-dependent kinase pathway and selenoenzyme (GPx) activities (1.1).

Finish data collection on the experiments designed to determine whether methylselenol causes colon cell cycle arrest and induces differential gene expression in the cyclin-dependent kinase pathway (1.3).

Perform bioinformatics search/study to understand gene data derived in the above cell culture experiments (1.1 &1.3).

Prepare extracts from plants (other than broccoli) and conduct the studies to determine other compounds that transcriptionally upregulate the TR ARE independent of selenium (2.3).

Complete analyses and report results of hypotheses 2.2-2.3.

Complete analyses and report results from the experiment designed to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression (2.6).

Complete enzyme and metabolite analysis (including RLGS) from experiment designed to study the interaction between selenium and folate (3.1).

Initiate RLGS on Ames dwarf mice and age-matched wild type controls (3.2).

Conduct developmental studies of tandem HPLC-ICP-MS analyses of plasma selenium (4.1).

Plan selenium intervention trial (human) (4.2).

Develop flow cytometric method for evaluating apoptosis in whole blood samples generated by selenium intervention study (4.2)

Initiate human selenium intervention trial, including recruitment, conducting on-site briefings for perspective subjects, clinical screening of applicants, consented enrollment of volunteers, randomization to treatment, performance of baseline measurements and commencement of blinded intervention. (4.2)

Year 3 (FY 2007)

Optimize the colon cell culture conditions and start a study to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway

(1.2).

Optimize the colon cell culture conditions and start a study to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells (1.4).

Determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorily upregulated (2.4).

Initiate preliminary experiments to test the hypothesis that simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) will severely damage the ARE-regulated antioxidant network and result in severe cellular damage (2.5).

Complete methylation assays and gene identification from RLGS in the selenium-folate and Ames dwarf mice studies (3.1 & 3.2).

Initiate RLGS study in Ames mice fed various forms of selenium (3.3).

Complete development of HPLC-ICP-MS method for selenium in plasma (4.1).

Conduct selenium intervention trial, including the completion of subject enrollment, conduct interim subject visits and attendant analyses, and compilation of baseline data for all subjects (4.2).

Year 4 (FY 2008)

Finish cell culture experiments designed to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway (1.2).

Finish cell culture experiments designed to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells (1.4).

Initiate studies to determine the role of differentially expressed genes, as discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium (1.5).

Complete the experiments designed to determine the effect of simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) (2.5).

Complete the studies designed to determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorily upregulated (2.4).

Complete analyses from the Ames dwarf mouse selenium study (3.3).

Initiate Ames dwarf aberrant crypt study (contingency).

Determine whether there is a relationship between methionine sulfoxide activity and/or expression and aberrant crypt formation in and aberrant crypt model (contingency for 2.6).

Complete validation of HPLC-ICP-MS analytical procedure and report results (4.1).

Complete selenium intervention trial; continue associated analytical work (4.2).

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2006

Year 5 (FY 2009)

Finish data collection and bioinformatics work on experiments designed to determine the role of differentially expressed genes, as discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium (1.5).

If resources are available, initiate experiments to determine the effect of other dietary antioxidants (other than sulforaphanes and selenium) in ARE-protein knockdown cell models.

Complete the Ames dwarf aberrant crypt study and related methionine sulfoxide studies.

Complete analytical work for selenium intervention trial (4.1).

Use HPLC-ICP-MS method to assess selenium status in samples from selenium intervention trial (4.2).

3a. List the milestones (from the list in Question 2) that were scheduled to be addressed in FY 2006.

1. Finish data collection on the Caco-2 cell culture studies designed to determine whether cellular selenium status regulates the cyclin-dependent kinase pathway and selenoenzyme (GPx) activities (1.1).

   Milestone Fully Met
2. Finish data collection on the experiments designed to determine whether methylselenol causes colon cell cycle arrest and induces differential gene expression in the cyclin-dependent kinase pathway (1.3).

   Milestone Fully Met
3. Perform bioinformatics search/study to understand gene data derived in the above cell culture experiments (1.1 & 1.3).

   Milestone Fully Met
4. Prepare extracts from plants (other than broccoli) and conduct the studies to determine other compounds that transcriptionally upregulate the TR ARE independent of selenium (2.3).

   Milestone Fully Met
5. Complete analyses and report results of hypotheses (2.2 - 2.3).

   Milestone Fully Met
6. Complete analyses and report results from the experiment designed to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression (2.6).

   Milestone Substantially Met
7. Complete enzyme and metabolite analysis (including RLGS) from experiment designed to study the interaction between selenium and folate (3.1).

   Milestone Substantially Met
8. Initiate RLGS on Ames dwarf mice and age-matched wild type controls (3.2).

   Milestone Fully Met

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2006

3a. 9. Conduct developmental studies of tandem HPLC-ICP-MS analyses of plasma selenium (4.1).

Milestone Substantially Met

10. Plan selenium intervention trial (human) (4.2).

Milestone Fully Met

11. Develop flow cytometric method for evaluating apoptosis in whole blood samples generated by selenium intervention study. (4.2)

Milestone Substantially Met

12. Initiate human selenium intervention trial, including recruitment, conducting on-site briefings for perspective subjects, clinical screening of applicants, consented enrollment of volunteers, randomization to treatment, performance of baseline measurements and commencement of blinded intervention. (4.2)

Milestone Fully Met

3b. **List the milestones (from the list in Question 2) that you expect to address over the next 3 years (FY2007, 2008 & 2009). What do you expect to accomplish, year by year, over the next three years under each milestone?**

FY 2007

Optimize the colon cell culture conditions and start a study to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway (1.2). This study, when completed, will help to determine how selenium affects the expression of cancer genes and, thus, will help us determine the mechanism of action of selenium.

Optimize the colon cell culture conditions and start a study to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells (1.4). This study, when completed, will help to determine whether selenium differentially affects tumor versus normal cells. Thus, if they are affected differently, we will have a better understanding of the mechanism of selenium's anticancer effect in vivo.

Determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorily upregulated (2.4). This study will demonstrate that antioxidant protection in a cell is conferred by multiple redundant systems, and knocking out only one system does not result in severe damage to the cell.

Initiate preliminary experiments to test the hypothesis that simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) will severely damage the ARE-regulated antioxidant network and result in severe cellular damage (2.5). This study will demonstrate that although loss of a single component of the antioxidant network will probably result in severe damage to the cell, loss of multiple components of the system will result in severe metabolic abnormalities. This preliminary study will be to determine which components of the system that contain an ARE and can be knocked down by siRNA have the most influence on oxidative status.

Complete methylation assays and gene identification from RLGS in the selenium-folate and Ames dwarf mice studies (3.1 & 3.2). These studies will help determine whether alteration in gene expression by means of DNA methylation is a mechanism through

which selenium is anticarcinogenic and whether the unique methionine metabolism in the Ames dwarf plays a role in the very low cancer rate found in these mice.

Initiate RLGS study in Ames mice fed various forms of selenium (3.3). This study, when completed, will help determine whether selenium acts synergistically in the Ames dwarf mouse to further reduce the incidence of cancer; the study will also be used to determine whether selenium affects methionine metabolism similar to the alteration of methionine metabolism in the Ames dwarf.

Complete development of HPLC-ICP-MS method for selenium in plasma (4.1). This work will result in a new, highly sensitive method for the quantitative determination of selenium in the major selenium-containing proteins in plasma, producing a "fingerprint" of selenium status that can be used in human and animal studies in the assessment of nutritional selenium status.

Conduct selenium intervention trial, including the completion of subject enrollment, conduct interim subject visits and attendant analyses, and compilation of baseline data for all subjects (4.2). This trial will be the first trial to use low doses of selenium to determine the quantitative relationship between the selenium intake and parameters of selenium status at equilibrium (i.e., after chronic exposure). These data will be the first to facilitate the development of algorithms for imputing the amounts of dietary selenium needed to support particular threshold levels of plasma selenium associated with reductions in chronic disease risk.

FY 2008

Finish cell culture experiments designed to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway (1.2). This study, when completed, will help to determine how selenium affects the expression of cancer genes and, thus, will help us determine the mechanism of action of selenium.

Finish cell culture experiments designed to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells (1.4). This study will help to determine whether selenium differentially affects tumor versus normal cells. Thus, if they are affected differently, we will have a better understanding of the mechanism of selenium's anticancer effect in vivo.

Initiate studies to determine the role of differentially expressed genes, as discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium (1.5). This study, when completed, will help confirm some of our in vitro findings in the in vivo model. Thus, it may result in finding biomarker candidate genes for the chemopreventive actions of selenium.

Complete the experiments designed to determine the effect of simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) (2.5). This study will utilize procedures developed FY 2007 and will provide definitive evidence that loss of multiple components of the system will result in severe metabolic abnormalities.

Complete the studies designed to determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorily upregulated (2.4). This study will use tools such as gene arrays to demonstrate that the loss of one component of the system is compensated for by upregulation of many other components of the system.

Complete analyses from the Ames dwarf mouse selenium study (3.3). This study will

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2006

help determine whether selenium acts synergistically in the Ames dwarf mouse to further reduce the incidence of cancer; the study will also be used to determine whether selenium affects methionine metabolism similar to the alteration of methionine metabolism in the Ames dwarf.

Determine whether there is a relationship between methionine sulfoxide activity and/or expression and aberrant crypt formation in and aberrant crypt model (contingency for 2.6). This study, when completed will help determine whether the mode of action of selenium anticarcinogenicity is through methionine sulfoxide activity and/or expression.

Complete validation of HPLC-ICP-MS analytical procedure for determination of selenium in plasma and report results (4.1). This method will produce a "fingerprint" of selenium status that can be used in human and animal studies in the assessment of nutritional selenium status.

Complete selenium intervention trial; continue associated analytical work (4.2). These data will be the first to facilitate the development of algorithms for imputing the amounts of dietary selenium needed to support particular threshold levels of plasma selenium associated with reductions in chronic disease risk.

FY 2009

Finish data collection and bioinformatics work on experiments designed to determine the role of differentially expressed genes, as discovered in previous cell culture studies, in mediating the anti-tumorigenic effect of selenium (1.5). This study, when completed may result in finding biomarker candidate genes for the chemopreventive actions of selenium.

If resources are available, initiate experiments to determine the effect of other dietary antioxidants (other than sulforaphanes and selenium) in ARE-protein knockdown cell models. Due to a critical SY vacancy, this milestone will not be completed.

Complete the Ames dwarf aberrant crypt study and related methionine sulfoxide studies. This study, when completed will help determine whether the mode of action of selenium anticarcinogenicity is through methionine sulfoxide activity and/or expression.

Complete analytical work for selenium intervention trial (4.1). These data will be the first to facilitate the development of algorithms for imputing the amounts of dietary selenium needed to support particular threshold levels of plasma selenium associated with reductions in chronic disease risk.

Use HPLC-ICP-MS method to assess selenium status in samples from selenium intervention trial (4.2). This application of this method will facilitate the determination of the impact of dietary selenium in the major components of the plasma selenium, thus demonstrating whether the new method can be useful in the assessment of nutritional selenium status.

4a. List the single most significant research accomplishment during FY 2006.

The elucidation of the mechanisms by which selenium regulates the cell cycle can lead to a better understanding of the nature of selenium's essentiality and its role in disease prevention. Methylselenol has been hypothesized to be a critical selenium

metabolite for anticancer activity. Methylselenol increased the protein levels of two antimestatic compounds TIMP-1 and TIMP-2) and inhibited the migration and invasion rate of fibrosarcoma cells in culture, decreasing the carcinogenic potential of activity of these tumor cells. Other cell culture work with human lymphocytes has shown that selenium deficiency inhibits cell cycle progression and decreases the mRNA expression of many cell cycle regulatory genes. Collectively, these results suggest that selenium is critical for human lymphocyte cell division, growth and prevention of cell death. Accomplishment aligns with Component 2: Relationship between diet, genetics and lifestyle and the risk for chronic disease.

4b. List other significant research accomplishment(s), if any.

The methionine sulfoxide reductase (Msr) system (comprised of MsrA and MsrB) is important in repairing oxidized proteins. The system is responsible for reducing methionine sulfoxide [Met(O)] to methionine. MsrB is a selenoprotein and reduces the Met-R-(O) isomer whereas MsrA (a non-selenoprotein) reduces the Met-L-(O) isomer. Typically, the racemic Dabsyl-derivative Met-R/S-(O) is used to assay the enzyme activity. However, some researchers use the individual S or R derivatives to distinguish MsrA from MsrB. We developed a new assay that uses capillary electrophoresis for determination of MsrA and MsrB enzyme activities and of Dabsyl-derivative purity. We found typical preparations of Dabsyl-Met-R-(O) are contaminated with the S-isomer (10-25%). Furthermore, the activity towards Dabsyl-Met S-(O) is less than 10% of the activity towards Dabsyl-Met-R-(O). Taken together, our data suggest that the selenoprotein MsrB accounts for the greatest percentage of enzyme activity in the Msr system and that researchers who use the racemic mixture Dabsyl-R/S-Met(O) to measure Msr activity need to realize that most of the associated activity is accounted for by the selenoprotein MsrB. This is important because most researchers ignore this fact and, therefore, results that are attributable to selenium have not been realized. Accomplishment aligns with Component 2: Relationship between diet, genetics and lifestyle and the risk for chronic disease.

The LoDoSe trial (4.2), which was planned and initiated in FY2006, constitutes the first human trial in which selenium doses less than 200 mcg/day have been used in non-deficient subjects. This is important for the reason that, while the 200 mcg/day dose has been found to reduce cancer risks in healthy Americans, further analyses suggested that selenium doses much less than that may be effective. That prospect has direct implications to the US food industry which can produce foods capable of providing 50-100 mcg selenium per day through geographic sourcing, or selenium-fortification. The results of the LoDoSe trial will provide the evidence base for the development of foods and food policy for reducing cancer risks by achieving target plasma selenium levels. Accomplishment aligns with Component 1: Nutrient requirements.

4c. List significant activities that support special target populations.

None.

4d. Progress report.

None.

5. Describe the major accomplishments to date and their predicted or actual impact.

This research is directly related to the ARS National Human Nutrition Research Plan 107, Component 2: Diet, Genetics, Lifestyle and the Prevention of Disease, and directly contributes to the accomplishment of ARS Strategic Plan Goal #4, Improve the Nation's Nutrition and Health.

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2006

Butyrate treatment inhibits the migration and invasion potential of tumor cells: Butyrate, a product of the bacterial fermentation of dietary fiber, has been hypothesized to be directly related to the lower risk of colon cancer. It is important to test and to characterize the invasive ability of tumor cells as affected by long exposure to low concentrations of butyrate. Our results demonstrate that prolonged and low-dose butyrate treatment (0.5 mmol/L butyrate, similar to a moderate fiber diet) inhibits pro-MMP-2 activation and tumor cell migration/invasion potential. Our data provide a possible mechanism for butyrate's anticarcinogenic properties. These data support the health claim of a high-fiber diet and provide a mechanism to link fiber and decreased cancer risk.

Thioredoxin reductase is regulated by sulforaphane: Thioredoxin reductase, a selenium-requiring protein, is transcriptionally regulated by sulforaphane from broccoli. Broccoli contains many other compounds that may inhibit cancer, and some of this inhibition may be mediated by turning on ARE containing enzymes such thioredoxin reductase. This hypothesis was investigated by adding compounds known to be in broccoli to cells in culture containing a reporter gene construct of thioredoxin reductase. Although ascorbic acid resulted in modest induction, the results convincingly demonstrated that sulforaphane was responsible for almost all of the ARE-mediated activation of thioredoxin reductase. This research provides a plausible mechanism to explain how broccoli consumption may inhibit cancer. Furthermore, this study provides a tool that can be used to screen various compounds to determine their cancer-fighting ability.

Glycine N-methyltransferase, a tumor susceptibility gene, is decreased in selenium deficiency: Determined that the activity of the enzyme glycine N-methyltransferase (GNMT) is decreased by selenium deprivation. GNMT, which has been shown to be a tumor susceptibility gene, is a regulator of tissue S-adenosylmethionine concentration, and in liver, is a major folate binding protein. Thus, GNMT may induce changes in tissue folate status resulting in chromosome breakage or abnormal DNA methylation. Second, GNMT is an enzyme participating in detoxification. In addition, GNMT may have a protective effect against the exposure to carcinogens by decreasing DNA adduct formation. Thus, the decrease in this enzyme may explain some of the effects of selenium deficiency. This study affirms the importance of the interaction between dietary selenium and folic acid and suggests that alterations in selenium status may affect folate status and vice versa. This may prove most important in the nutrition of those humans who may have low folate and low selenium status. That is, supplementation of one without the other may be more detrimental than beneficial.

Transsulfuration is markedly enhanced in the long-lived Ames dwarf mouse: Shown by using tracer studies that transsulfuration is markedly enhanced in the Ames dwarf mouse. The Ames dwarf mouse lives substantially longer and has a lower incidence of cancer than the wild type. This tracer study, along with real-time RT PCR findings of genes associated with methionine metabolism, provides a plausible mechanism for the increased glutathione found in the dwarf mice. This supports the hypothesis that these mice have enhanced oxidative defense capabilities. This study gives insight to what mechanisms in animals are important in aging and in decreasing the risk of cancer; similar mechanisms (and how nutrition affects them) could then be studied in humans.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2006

Information on how trace elements (specifically selenium) affect mechanisms related to carcinogenesis was presented at numerous workshops and scientific meetings.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).**

Eight presentations/publications were made: three Grand Forks Herald articles, "B12: It's more Important than You Think," "Diet, Lifestyle Play Role in Preventing Cancer," and "Diet High in Folic Acid Can Help Fight Disease;" and talks at the East Grand Forks Senior Citizen's Center entitled "Food for Thought" and "What Is Selenium?;" a seminar at South Dakota State University "The Role of Selenium in Reducing Cancer Risk;" a talk at the Grand Forks Rotary Club "The Role of the USDA in Researching Questions of Diet and Health;" and a lecture at the 9th International Symposium on Metal Ions in Biology and Medicine, Lisbon, "Determining Healthful Intakes of Selenium."

Scientific Publications: **Log 115:**

1. Combs, Jr., G.F. 2006. Drinking water as a source of mineral nutrition. In: Institute of Medicine of the National Academies, editors. Mineral Requirements for Military Personnel: Levels Needed for Cognitive and Physical Performance During Garrison Training. Washington, DC:National Academies Press. p. 295-304. 0000181954
2. Stranges, S., Marshall, J.R., Trevisan, M., Natarajan, R., Donahue, R.P., Combs, G.F., Farinaro, E., Clark, L.C., Reid, M.E. 2006. Effects of selenium supplementation on cardiovascular disease incidence and mortality: Secondary analyses in a randomized clinical trial. American Journal of Epidemiology. 163:694-699. 0000181077
3. Uthus, E.O., Brown-Borg, H.M. 2006. Methionine flux to transsulfuration is enhanced in the long living Ames dwarf mouse. Mechanisms of Aging and Development. 127:444-450. 0000188688
4. Uthus, E.O., Ross, S., Davis, C.D. 2006. Differential effects of dietary selenium (se) and folate on methyl metabolism in liver and colon of rats. Biological Trace Element Research. 109:201-214. 0000175013
5. Zeng, H., Briske-Anderson, M.J., Idso, J.P., Hunt, C. 2006. The selenium metabolite methylselenol inhibits the migration and invasion potential of HT1080 tumor cells. Journal of Nutrition. 136:1528-1532. 0000189362
6. Chang, W.P., Combs, G.F., Scanes, C.G., Marsh, J.A. 2005. The effects of dietary vitamin E and selenium deficiencies on plasma thyroid and thymic hormone concentrations in the chicken. Developmental and Comparative Immunology 29:265-273. 0000196797
7. Combs, Jr., G.F. 2005. Importance of selenium in human nutrition. Agrifood Research Reports 69. Proceedings, Twenty Years of Selenium Fertilization. p. 60-70. 0000184741
8. Brown-Borg, H.M., Rakoczy, S.G., Uthus, E.O. 2004. Growth hormone alters components of the glutathione metabolic pathway in Ames dwarf mice. Annals of the New York Academy of Sciences. 1019:317-320. 0000195345
9. Patterson, B., Wasteny, M., Combs, G.F., Brindak, M., Patterson, K.K., Veillon, C., Taylor, P., Levander, O.A. 2006. Selenium metabolism in humans: Response of kinetic pools in plasma to 2 yr supplementation. The FASEB Journal Book of Abstracts, Volume 20, No. 5, p. A1069:673.11. 0000187164
10. Uthus, E.O., Ross, S. 2006. Dietary selenium (Se) but not folic acid (FA) affects the activity and message of rat liver betaine homocysteine 0000187382

Project Number: 5450-51000-036-00D **Accession:** 0408616 **FY:** 2006

methyltransferase (BHMT)[abstract]. Journal of Federation of American Societies for Experimental Biology. 20(4):A429.

11. Zeng, H., Briske Anderson, M.J., Idso, J.P., Hunt, C. 2006. Selenium metabolite methylselenol inhibits migration and invasion potential of HT1080 tumor cells [abstract]. FASEB J. 20(5):A1011. **0000187192**

Approved: BLACKBURN WILBERT H **Date:** 09/29/2006

Project Number: 5450-51000-036-01R Accession: 0406009 FY: 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: DAVID M KLURFELD Principal Investigator: ERIC O UTHUS
Start Date: 07/15/2002 Term Date: 06/30/2007

National Programs: 107 N Human Nutrition

Title: DETERMINATION OF NUTRIENT EFFECTS ON CANCER SUSCEPTIBILITY ON EPIGENETIC PROCESSES IN ANIMAL MODELS

Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? No
Terminate in Two Months? No

Agreement Number: 02-5450-2-0217

Organization Name: NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES

Progress and Outcomes:

4a. List the single most significant research accomplishment during FY 2006.

None.

4b. List other significant research accomplishment(s), if any.

None.

4c. List significant activities that support special target populations.

None.

4d. Progress report.

This report serves to document research conducted under an interagency reimbursable agreement between ARS and the National Cancer Institute. Additional details of research can be found in the report for the parent project 5450-51000-036-00D.

The purpose of this agreement is to examine the interactive effects of dietary selenium and folate on cancer susceptibility, global and gene specific DNA methylation, DNA methyltransferase activity, and one-carbon metabolism using animal models. This work supports that done in 5450-51000-031-00D, Role of Selenium on Gene Expression, Cell Cycle and Molecular Mechanisms in Cancer Risk.

A central hypothesis to this research is that selenium affects DNA methylation and, hence, gene expression. There is a commercial vendor who supplies methylation arrays. These arrays are designed to look at promoter region methylation in 82 human genes, many of which are either oncogenes or tumor suppressor genes. We cultured Caco-2 cells with media containing 0, 3.9, 7.8, 15.6, or 250 nM selenium (as selenocysteine). These cells were then collected for DNA or RNA isolation. DNA was used in conjunction with the methylation arrays. We found several genes that were differentially expressed based on selenium content of the media. One of these genes was VHL (von Hippel-Lindau tumor suppressor). This gene appeared downregulated in cells cultured with no added selenium (compared to cells cultured in 15.6 nM Se). Real time RT PCR confirmed the downregulation as determined by mRNA expression. Analysis of individual CpG's for methylation status is presently underway. These results show that selenium status can affect DNA methylation and, hence, gene expression. Specifically, these results show that a tumor suppressor gene, VHL, as a result of promoter region methylation, is downregulated by deficient selenium. This

Project Number: 5450-51000-036-01R **Accession:** 0406009 **FY:** 2006

may be one of the mechanisms why increased cancer/cancer susceptibility is seen with selenium deficiency.

Our previous work showed that selenium status in rats affects plasma and tissue concentrations of homocysteine and that low dietary selenium results in a decreased activity of liver betaine homocysteine methyltransferase (BHMT). BHMT is one of two enzymes that catalyzes the remethylation of homocysteine to methionine. The other pathway for removal of homocysteine is the transsulfuration pathway. Researchers from Spain found, in humans, that selenium status may be a more powerful indicator than folate in predicting plasma homocysteine. They suggested that this could be the result of selenium on BHMT. We found that in selenium-deficient mice, plasma homocysteine was significantly reduced. In contrast to rats, however, BHMT (activity or mRNA) was not affected by dietary selenium. Another enzyme that can affect homocysteine metabolism is glutamate cysteine ligase (Gcl). Gcl catalyzes the rate limiting step in the synthesis of glutathione and is part of the transsulfuration pathway. Gcl has two subunits, the catalytic subunit Gclc and the modifier subunit Gclm. In the rat and mouse, liver Gclc mRNA is significantly increased by selenium deficiency. Gclm mRNA is also increased by selenium deficiency in the rat but selenium has no effect on Gclm in mouse. These results indicate that there are species differences in how selenium affects homocysteine metabolism. Further, the results suggest that BHMT may not be the key player in regulation of plasma homocysteine.

Progress was made on establishing the methodology to use the Big Blue rodent model to evaluate spontaneous mutation rates as affected by diet. This model uses transgenic rats that carry a chromosomally integrated lambda bacteriophage containing E. coli lacI gene as a target for mutation. Lambda phage are rescued by in vitro packaging and screened for mutations. This model will be used to determine whether dietary selenium (form and dose) affect spontaneous mutation rates. A pilot study to develop the required procedures was successfully conducted. The laboratory portion of a full scale study has been completed. This study will ascertain (compared to selenium adequacy) whether selenium deficiency or supranutritional selenium affect spontaneous mutations.

Scientific Publications: **Log 115:**

Approved: CHANDLER LAURENCE D **Date:** 09/26/2006

Project Number: 5450-51000-036-02R Accession: 0410110 FY: 2006
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: DAVID M KLURFELD Principal Investigator: GERALD F COMBS
Start Date: 09/01/2005 Term Date: 09/30/2007

National Programs: 107 N Human Nutrition

Title: SELENIUM NUTRITION IN HUMANS: PREDICTING DIETARY SELENIUM NEEDS TO ACHIEVE TARGET BLOOD SELENIUM LEVELS

Period Covered From: 10/ 2005 To: 9/ 2006 Final Report? No
Terminate in Two Months? No

Agreement Number: 05-5450-5-0330

Organization Name: NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES

Progress and Outcomes:

4a. List the single most significant research accomplishment during FY 2006.

None

4b. List other significant research accomplishment(s), if any.

None

4c. List significant activities that support special target populations.

None

4d. Progress report.

This report serves to document research conducted under an interagency reimbursable agreement between ARS and National Cancer Institute of the National Institutes of Health. Additional details of research can be found in the report for the parent project 5450-51000-036-00D.

This collaboration planned and implemented the first clinical intervention trial using nutritional doses of selenium, the "LoDoSe trial", which was initiated in FY2006 and will continue through FY2008. This trial will provide the fundamental information to allow the projection of daily dietary selenium needs from plasma selenium levels found to be thresholds for such health effects as minimalization of cancer risks in healthy Americans, which prospect has direct implications to the US food industry which can produce foods capable of providing 50-100 mcg selenium per day through geographic sourcing, or selenium-fortification. This year, more than 300 adult men and women volunteers were recruited and screened, and a total of 213 subjects were enrolled in the study. This comprises 89% of our enrollment target of 240 total subjects. In addition, a new methodology was developed for assessing the degree cellular resistance to oxidative stress-induced programmed cell death (apoptosis), using high-volume flow cytometric analysis of whole blood samples. This method may have special utility for both nutritional and toxicological evaluations using minimally invasive techniques.

Scientific Publications: Log 115:

Approved: CHANDLER LAURENCE D Date: 09/06/2006